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SYMPOSIUM

Chronic Fungus Infections

Special Editor

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INTRODUCTION TO SYMPOSIUM ON FUNGUS INFECTIONS

FUNGUS infections are characteristically chronic and should be discussed in a journal which disseminates knowledge concerning the chronic diseases which inflict intense and prolonged suffering on mankind.

All of the fungus organisms which infect man were known before World War II, but they were usually regarded as rare and exotic agents of no practical importance either to the general practitioner or the internist. Intensive studies during World War II revealed that fungus infections were widely distributed throughout the population and even occurred in epidemic form under certain conditions. The use of the broad spectrum antibiotics has reduced the relative

importance of the common bacterial infections and has increased the relative importance of the fungus diseases. Certain fungus infections have increased in number and severity since the introduction of the broad spectrum antibiotics, apparently as a result of a temporary destruction of the normal harmless bacterial flora which usually holds the fungus flora in check. The widespread, and often uncritical, use of the corticosteroid hormones has increased the number of infections with fungi of limited pathogenicity which normally infected man with difficulty. Local or limited infections, with fungi of greater pathogenicity, may suddenly become aggravated and disseminated throughout the body by unwitting therapy with the corticosteroid hormones.

Some of the fungus infections such as actinomycosis, nocardiosis, cryptococcosis, sporotrichosis, candidiasis, and the dermatomycoses of hair, skin, and nails occur throughout this country and throughout the world. Others such as coccidioidomycosis, histoplasmosis, and blastomycosis occur primarily, if not exclusively, in certain geographic areas. Physicians living in the endemic areas of these three mycoses have learned about them on the hard road of experience, but it is even more important that physicians practicing in other parts of the country acquire a working knowledge of these three diseases. Travel is almost continuous throughout this country, and the knowledge that a patient has visited an endemic area recently or even a number of years ago may give the clue to the correct diagnosis.

The dermatophytes can be transmitted from man to man. Some of them are acquired from dogs, cats, or cows, while others are transmitted directly from man to man. The fungi which cause systemic infections, are fortunately not transmitted from man to man, and strict isolation of the patient is not necessary.

Actinomycosis, candidiasis, and geotrichosis are human pathogens in the same sense as staphylococcal, pneumococcal, streptococcal, and meningococcal infections. They are passed around from man to man but do not always cause clinical disease. Most of the systemic infections of man are exogenous and are acquired from the soil or from organic dusts. Nocardiosis, cryptococcosis, blastomycosis, sporotrichosis, coccidioidomycosis, histoplasmosis, and fungi of minor importance are characterized by an exogenous origin.

Diagnosis and therapy has been emphasized in this symposium, but the correct diagnosis is rare unless the disease is suspected. The systemic mycoses are as polyphasic in their manifestations as syphilis, tuberculosis, or neoplasms, and they should be considered in the differential diagnosis of every obscure chronic infection.

The general treatment for the systemic mycosis resembles the general treatment for tuberculosis, namely, bed rest, reassurance, and a good diet fortified with vitamins. Surgical drainage is indicated in some instances, and under certain conditions excisional surgery is curative.

Unfortunately, penicillin and the broad spectrum antibiotics are not only useless but may be harmful. There are two exceptions to this statement; actinomycosis is curable with either sulfonamides or penicillin, and nocardiosis is curable with sulfonamides but not with penicillin. Neither disease, however, will respond

to a short course of treatment. Hydroxystilbamidine is an effective specific drug for the treatment of blastomycosis although some failures do occur. Potassium iodide usually cures sporotrichosis and is helpful as a supplementary treatment of a number of other mycotic infections. We need effective specific drugs for the treatment of coccidioidomycosis, histoplasmosis, cryptococcosis, and candidiasis. A certain degree of limited success has been achieved with some of the newer fungistatic drugs, and these will be discussed in the separate contributions to the symposium.

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VOL.
5
1957

ACTINOMYCOSIS AND NOCARDIOSIS

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REGARDLESS of certain gross similarities between the two diseases and an even closer morphologic resemblance between the causal organisms, actinomycosis and nocardiosis must be regarded as separate and distinct diseases. Both infections are caused by closely related members of the order actinomycetales (Fig. 1), a group of organisms intermediate between the true bacteria and more complex fungi. The distinguishing criterion of the entire order is the propensity for filamentous growth with true branching, a collection of branching

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Order Actinomycetales

MYCELIUM RUDIMENTARY OR ABSENT

1. Mycobacteriaceae
   - Mycobacterium

TRUE MYCELIUM PRODUCED

2. Actinomycetaceae (Fragment into bacillary and coccoid elements)
   - Actinomyces
     - Anaerobic and nonacid-fast—*Actinomyces bovis*
   - Nocardia
     - Aerobic
       - Acid-fast—*Nocardia asteroides*
       - Nonacid-fast
3. Streptomycetaceae (Do not fragment)
   - Streptomyces
   - Micromonospora

Fig. 1. (Modified after Waksman and Henrici.[1])

filaments being known as the mycelium. Among the mycobacteriaceae the mycelium is usually rudimentary or absent, although the occurrence of short branching forms of Mycobacteria is well recognized. Members of this genus are acid-fast. The rest of the actinomycetales form a true mycelium but differ in that the actinomycetaceae fragment into bacillary and coccoid forms whereas the streptomycetaceae do not. The streptomycetaceae can be differentiated readily from other actinomycetales by the characteristic colonial features of a tough coherent vegetative mycelium, unusually elaborate aerial hyphae and lack of fragmentation. None of the streptomycetaceae are acid-fast.

The actinomycetaceae include the Actinomyces, represented primarily by *Actinomyces bovis*, and the Nocardia, of which the outstanding example is *Nocardia asteroides*. Although both are gram-positive and produce delicate branching filaments (hyphae) approximately 1 μ in diameter, they are easily identified by their different growth characteristics. Thus the Actinomyces are anaerboic or microaerophilic growing well at incubator temperature but poorly if at all at temperatures below 30° C. The Nocardia, on the other hand, grow aerobically on all common laboratory media at both room and incubator temperatures. Moreover, the Actinomyces are nonacid-fast whereas some of the Nocardia, notably *N. asteroides*, exhibit varying degrees of acid-fastness. While it seems absurd that members of these two genera should be confused in cultures, this is not uncommon and until recently it was popular to lump the diseases produced by both under the title actinomycosis, a fact undoubtedly responsible in part for the supposed rarity of nocardiosis. In some places the diagnostic implications of an aerobic actinomycete are still not appreciated.

VOL.
5
1957

True branching of many filaments (Fig. 2) is an observation usually sufficient to separate a Nocardia from the Mycobacteria, but not always since *N. asteroides* may appear in the fragmented bacillary form without showing any evidence of branching (Fig. 3). In addition, *N. asteroides* may be almost as acid-fast as the tubercle bacillus and it may be impossible to differentiate the two by Ziehl-Neelsen stained smears alone, particularly when dealing with Nocardia in fresh exudate. Ordinarily, however, the degree of acid-fastness of the Nocardia is less than that of the Mycobacteria, many strains of *N. asteroides* tending to lose the carbolfuchsin unless decolorized for appreciably shorter periods of time or with weak acid rather than acid alcohol, while others attain moderate acid-fastness only after growth on litmus milk. Growth at room temperature will rule out *M. tuberculosis*, but does not eliminate a saprophytic Mycobacterium.

Actually, the greatest difficulty may arise from attempts to distinguish between saprophytic Mycobacteria and *N. asteroides*. Mycobacteria do not produce an aerial mycelium under any cultural conditions, hence any acid-fast aerobic organism producing an aerial mycelium is a Nocardia. Nevertheless, the growth requirements of each, the time necessary for colonial growth, their chromogenicity, staining properties, and microscopic appearance may all be quite similar, so that it is not unusual to see nocardial cultures discarded in the erroneous notion that they represent saprophytic Mycobacteria. For such occasions animal pathogenicity may be invaluable for identifying the organism in question. In fact, it is our own feeling that pathogenicity for guinea pigs or chick embryos should be a requirement for inclusion in the species asteroides. This, of course, is in marked contrast to *A. bovis* which, like the saprophytic Mycobacteria, exhibits a nearly complete lack of pathogenicity for ordinary laboratory animals.

ACTINOMYCOSIS

Historical Highlights.—Actinomycosis was first described in 1877, when Bollinger[6] reported the cervicofacial form of the disease ("lumpy jaw") in cattle, giving credit to Harz for demonstrating the organism in pus from these animals. Apparently impressed with the radiating margin of the actinomycotic granule

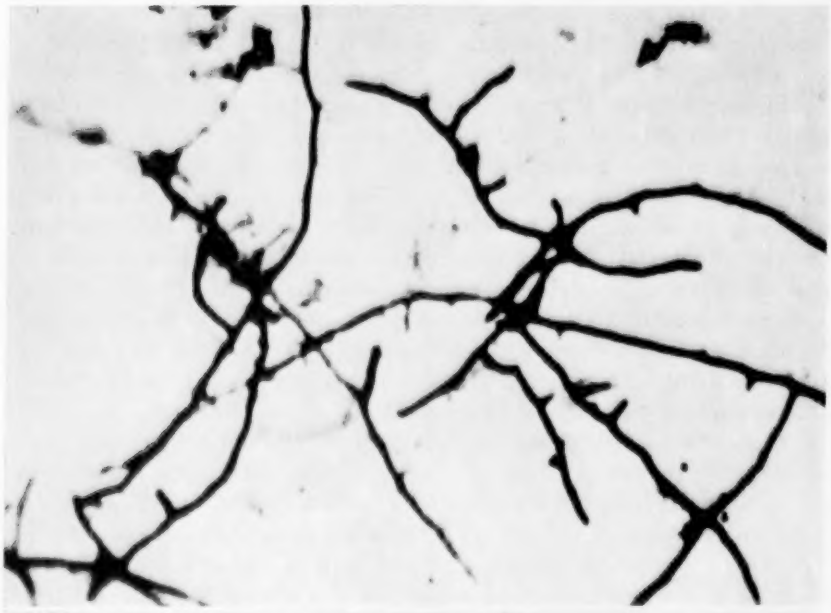

Fig. 2.

Fig. 3.

Fig. 2.—Branching Filaments. The above smear is from a culture of *N. asteroides*, but is morphologically indistinguishable from *A. bovis*. Both are gram-positive, branching, filamentous fungi measuring about 1 μ in diameter. Gram stain.

Fig. 3.—Fragmentation into bacillary forms. Another smear from a culture of *N. asteriodes* showing the pronounced tendency for fragmentation into bacillary and coccoid elements. This is quite typical of both Nocardia and Actinomyces and accounts for their being mistaken for bacilli, especially diphtheroids. Gram stain.

("sulfur granule") and recognizing its fungal origin, Harz himself published a subsequent report[7] in which he applied the term Actinomyces (ray-fungus) to the organism and called the disease actinomycosis. Soon afterwards Israël[8] cited the first human case (1878) and within the short space of 7 years collected an additional 37 cases.[9] Finally, in 1891, Wolff and Israël[10] described the cultural characteristics of the fungus and in particular its anaerobic growth.

Considerable confusion arose from Bostroem's claim (1891)[11] that the organism was actually aerobic and a rather prevalent saprophyte, considering the ease with which he recovered it from grain and straw. Such a theory was attractive, for it correlated well with the apparent predominance of the disease in farmers and cattle. Actinomycosis became known as a rural disease. The risk of infection was thought to be greater at harvest time and the habit of chewing straw was often blamed as the cause. It was almost 15 years before Wright tried to rectify this error by a report of 13 human and 2 bovine cases from all of which he had recovered anaerobic or microaerophilic Actinomyces.[12] Later investigators confirmed the work of Wolff, Israël, and Wright. Nevertheless, either through Bostroem's early influence or due simply to the nearly identical morphology of the two organisms, there persisted for many years a great deal of apathy about making any distinction between the aerobic and anaerobic actinomycetes. Far more emphasis was placed on the presence of "sulfur granules" (Fig. 4) than on cultural characteristics for the diagnosis of actinomycosis. Only in recent years has there been any appreciable improvement in this attitude.

Epidemiology.—Actinomycosis is a chronic granulomatous disease exhibiting an impressive aptitude for suppuration, abscess formation, and eventually the development of multiple draining cutaneous fistulas with extension of the process by contiguity without regard for tissue planes. Judging from the world-wide distribution of reported cases, actinomycosis stands as the most ubiquitous of the deep mycoses and is often referred to as the commonest; although if one considers only the United States, it is outnumbered by both histoplasmosis and coccididioidomycosis, and possibly by blastomycosis as well. Persons of any age may be affected but especially young adults. There appears to be no racial predilection. The incidence in males is roughly twice that in women, and farmers tend to be infected more often than those in any other occupation. The latter point, namely the higher incidence among agricultural workers, has been disputed by some who contend that the disease is as common in urban as in rural areas.[13] However, an analysis of all cases of actinomycosis seen in Scotland over a 13-year period[14] indicates that farmers are more apt to contract the disease than nonagricultural workers and that residents of larger towns are much less frequently infected than residents of rural areas.

Pathogenesis.—In view of its apparent predilection for farmers, the logical impulse is to incriminate some sort of occupational exposure to the fungus; but contrary to popular belief, anaerobic Actinomyces have not been found in nature which is a decided contrast to the aerobic actinomycetes which are readily recovered from soil. On the other hand, the organism's natural habitat is the human mouth where it has been cultured repeatedly from gums, carious teeth,

and tonsillar crypts.[15-26] Thus, despite the higher incidence of actinomycosis among agricultural workers, there is no basis for the traditional idea that infection is related to grass and straw, and it is now widely held that most cases of actinomycosis are endogenous in origin. Pieces of straw purportedly found within cervicofacial actinomycotic abscesses are better looked upon as traumatic agents, comparable to a tooth extraction that permits naturally occurring oral Actinomyces to gain access to the tissues. One might argue more logically that the greater incidence among farmers may be attributable to their poorer than average oral hygiene.

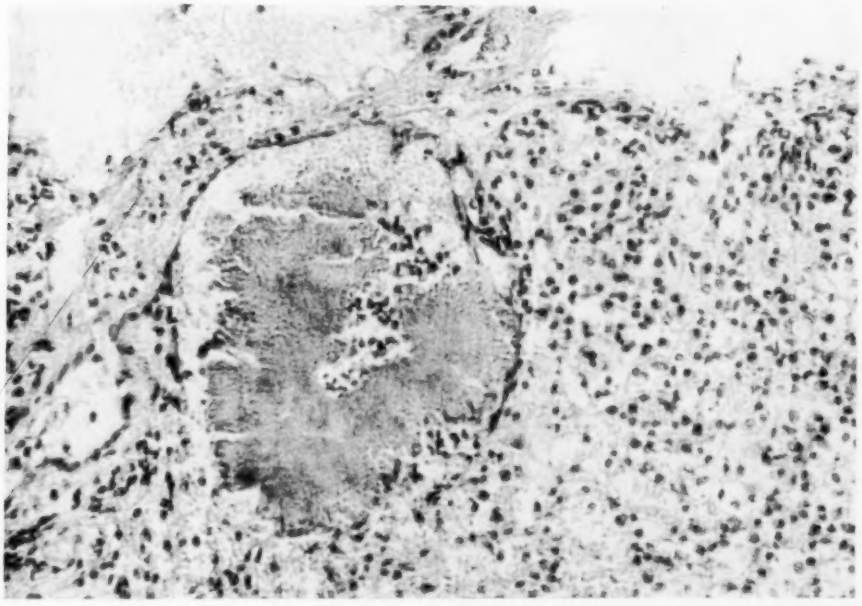

Fig. 4.—Sulfur granule. Typical actinomycotic granule seen in tissue obtained from biopsy of the wall of a draining cutaneous sinus. Such granules are characteristic but not pathognomonic of actinomycosis, occurring also, though much less frequently, in nocardiosis. Seen also in exudate where they usually possess a yellowish color, hence the term, sulfur granule. Hematoxylin and eosin stain.

Just why an organism exhibiting as little animal pathogenicity as *A. bovis* should be capable of assuming such virulence for man is difficult to understand. Attempts at reproducing the disease by the inoculation of laboratory animals with pure cultures of *A. bovis* alone have seldom been successful. It is probably significant that certain bacteria are found routinely in association with anaerobic Actinomyces recovered from cervicofacial lesions. Moreover, these other organisms have been shown to possess pathogenicity[23,24] which in turn prompted Holm[25] and later Glahn[26] to suggest that these bacteria are as essential as the Actinomyces for the development of progressive actinomycosis. The exact significance of these findings is not yet established, however.

Mycology.—Until recently there was general agreement that anaerobic Actinomyces obtained from "lumpy jaw" of cattle was identical with strains

recovered from human infections. The validity of this assumption was questioned by Erikson[27] who showed that most strains isolated from human lesions produced rough cauliflower-like colonies on agar and ball-like masses in broth. In contrast, the majority of bovine strains produced smooth colonies on agar and diffuse growth in broth. Finer cultural distinctions were also noted. Erikson termed the human species *Actinomyces israeli;* the bovine, *Actinomyces bovis*. Thompson[28] has subsequently corroborated this work although pointing out that these species are not host specific, some bovine infections definitely resulting from *A. israeli* and a few cases of human actinomycosis probably being due to *A. bovis*. Nevertheless, the two organisms are so closely related that most current descriptions of human disease continue to use the broad species designation, *A. bovis*. This is quite satisfactory for the purposes of this presentation, and hereafter no attempt will be made to distinguish between the two.

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957

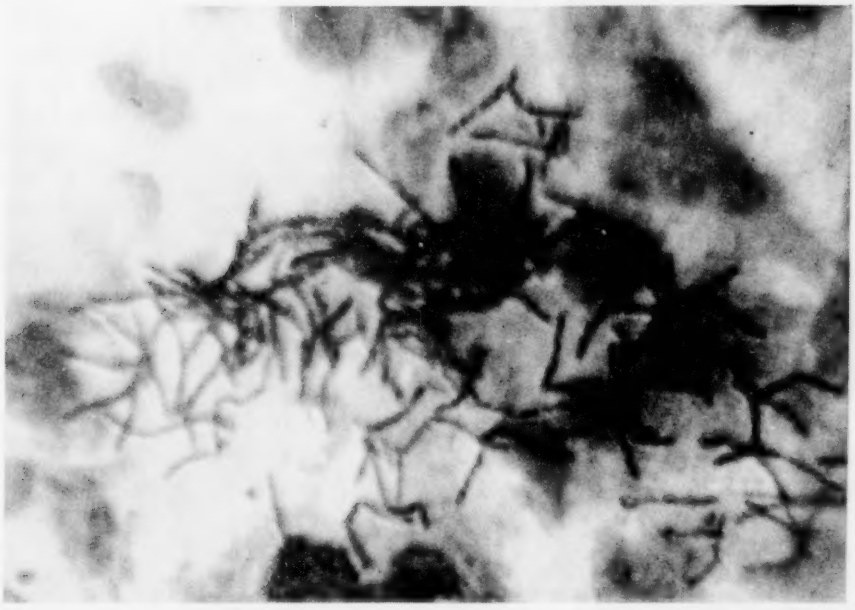


Fig. 5.—Actinomyces bovis in sputum. Clump of gram-positive, short branching filaments (hyphae) in sputum stained by Gram's method. Their appearance is consistent with either Actinomyces or Nocardia; in this case *A. bovis* was recovered by anaerobic culture.

A. bovis is an anaerobic or microaerophilic, gram-positive, nonacid-fast organism composed of long, slender, branching filaments. Organisms can be demonstrated in smears of sputum, pus, and exudate by means of the Gram stain, but because of its tendency to fragment (Fig. 5) Actinomyces are easily confused with diphtheroids. In infected material Actinomyces are often found in the form of flakes or "sulfur granules." These can be crushed beneath a cover slip and examined directly and also by the Gram stain. They are comprised of narrow, intertwined filaments slightly less than one micron in diameter.

At the periphery of the granules the filaments may be sheathed or clubbed, thus providing the radiate formation that accounts for the name of the organism ("ray fungus"). It is important to emphasize that many fungi, among them the Nocardia, Monosporium, etc., and some staphylococci ("botryomycosis") may produce granules resembling those produced by *A. bovis*.[29,30]

From the point of view of cultural characteristics *A. bovis* is one of the more distinctive fungi. It is the only one causing systemic infection that does not grow at room temperature on ordinary laboratory media under aerobic conditions. Its anaerobic quality has already been referred to and this, plus its failure to grow at room temperature, stands in sharp contradistinction to *Nocardia asteroides*.

In handling uncontaminated specimens such as pus obtained by needle puncture of a closed abscess or empyema, *A. bovis* may be recovered by inoculation of Brewer's thioglycollate glucose broth or veal infusion glucose agar shake tubes at 37° C. After 4 to 7 days suspicious colonies can be fished out and examined microscopically.

Contaminated specimens such as sputum, purulent drainage, or scrapings from sinus tracts should be studied by the method of Rosebury, Epps, and Clark.[22] Plates of Bacto^{Rx} brain-heart infusion agar are serially streaked with suspicious material by means of a sterile bent glass rod. By placing a loopful of sterile broth on the agar the distribution of the inoculum will be facilitated. These plates are then incubated at 37° C. in Brewer anaerobic jars containing 5 per cent carbon dioxide. By this method rough, starchy white or grayish colonies, 1 to 3 mm. in diameter, appear usually within 4 to 7 days. The colony is adherent to the agar and often comes away in one piece and leaves a pitted mark behind. Microscopic examination confirms the nature of the mycelial clusters. Because *A. bovis* is susceptible to penicillin, this antibiotic should not be used as a bacterial suppressant in culture media.

A. bovis possesses little or no pathogenicity for most laboratory animals and even by the injection of certain bacteria and foreign bodies together with pure cultures of Actinomyces, progressive infection has not been regularly produced. However, progressive disease has been reported in young mice by the intraperitoneal injection of pure cultures suspended in 5 per cent hog gastric mucin,[31] and in hamsters by the intraperitoneal injection of pure cultures alone.[32] While chick embryos of 7 to 9 days' incubation are susceptible to inoculation of the yolk sac with *A. bovis*, they are not killed by the infection. In the senior author's experience this method provides no advantage over others for the isolation of *A. bovis*.

Because of the organism's proclivity for saprophytic growth in the oral cavity, throat, and tracheobronchial tree, the repeated finding of *A. bovis* in the sputum is insufficient evidence to prove that the pulmonary disease seen in x-rays is necessarily related to this fungus. Kay,[33,34] for example, found this organism in the sputum of a large number of patients with nonspecific chronic bronchial disease without any evidence of pulmonary actinomycosis. Indisputable proof of its pathogenicity can be provided by the microscopic demonstration of the organism invading tissue, yet even the recovery of *A. bovis* from

a closed abscess or empyema demands a careful search for a more significant organism before attributing the disease in question to this cause alone.

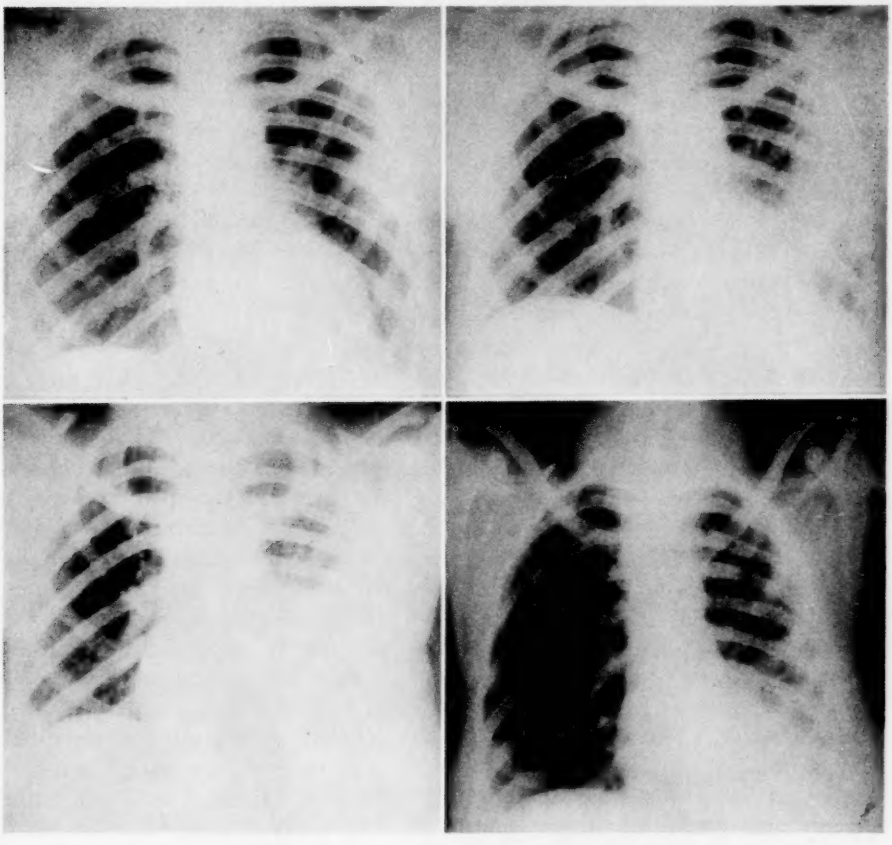


Fig. 6.—Actinomycosis cured by sulfonamides. Twenty-six-year-old Negro woman treated by prolonged bed rest (prestreptomycin era) because of a soft exudative infiltration in the third left anterior interspace (*A*). Lesion slowly extended to involve the pleura and chest wall (*B* and *C*). Subcutaneous abscesses appeared on the chest wall and later led to multiple draining sinuses. Biopsy taken at the time of surgical drainage disclosed many actinomycotic granules, thus affording presumptive evidence of actinomycosis. After 7 months' treatment with sulfonamides all sinuses closed and marked radiographic improvement ensued (*D*). She remains well 12 years later.

Clinical Spectrum.—It is customary to divide actinomycotic infections into 3 types, viz., cervicofacial, abdominal, and thoracic. Cope's elaborate review of 1,330 cases of actinomycosis showed over half to be cervicofacial, about one-quarter to be abdominal, 15 per cent thoracic, and a small percentage localized to other sites.[36] Most reported series of cases correlate fairly well with these figures, although an interested and efficient dental department has

been known to augment the percentage of cervicofacial infections[13] and in the Johns Hopkins series abdominal involvement predominated.[37] Part of the reason for the disproportionately high reported incidence of infections about the jaw must stem from the easier accessibility of such infections to discovery and diagnosis.

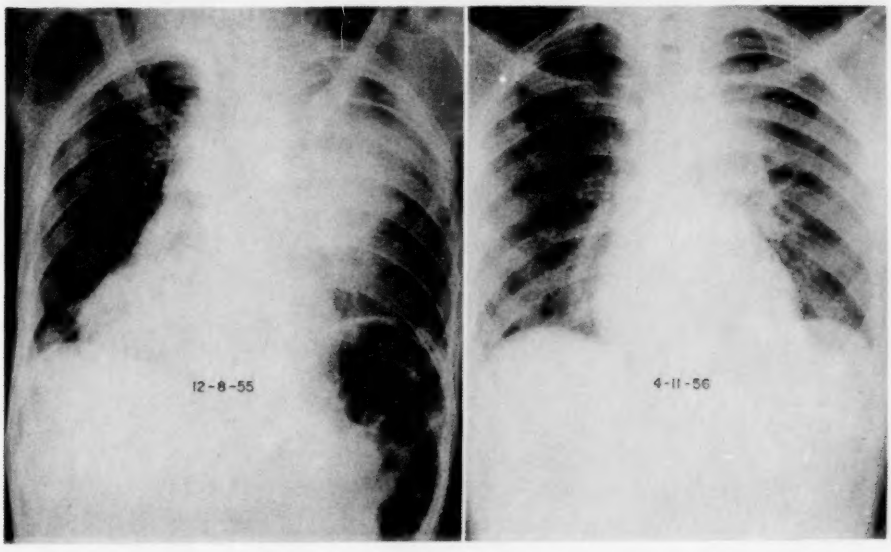
Cervicofacial actinomycosis is usually secondary to some antecedent trauma such as a tooth extraction or fractured jaw. Ordinarily it affects first the lower jaw in the vicinity of the angle of the mandible but later involves a larger surrounding area of the neck and face. The resultant swelling and induration has been picturesquely labeled "lumpy jaw," a term applied rather loosely in veterinary medicine to bovine actinomycosis and similar infections about the jaw. The overlying skin often has a violaceous hue and typically presents one or more draining sinuses which may on occasion have extensive communication with additional sinuses in the back, chest, or abdomen. Pain is seldom prominent and the patient usually appears well otherwise. The prognosis has always been more favorable in cervicofacial actinomycosis and since the availability of effective drug therapy the outlook has become most optimistic, provided of course that the etiology is recognized.

Thoracic actinomycosis is a great deal more diversified. It presumably results from the aspiration of Actinomyces-laden material from the mouth into the tracheobronchial tree where, with the symbiotic aid of accompanying bacteria plus bronchiolar occlusion secondary to simultaneously aspirated particulate matter, a pulmonary infection is established. In a few instances infection was thought to have arisen via the esophagus with resultant mediastinal and secondary pleuropulmonary involvement. Early in the disease because of the low virulence of the organism, there are notoriously few symptoms and seldom any systemic response other than mild fever. For these reasons, diagnosis is usually made at a late stage. Even then the symptomatology, much like that of tuberculosis, is likely to be unimpressive, especially nowadays when the indiscriminate use of penicillin for any pulmonary infection may be enough to abort the disease temporarily and thus becloud the issue.[38] There is usually a mild cough productive of scanty mucopurulent sputum, but hemoptysis is uncommon. Eventually there almost invariably ensues some involvement of the pleura and chest wall. Cutaneous fistulas are commonplace. The disease is slowly progressive and ultimately accompanied by high fever, night sweats, weight loss, and general debility. As might be anticipated from the suppurative nature of actinomycosis, there is generally a rather marked leukocytosis. Because of the propensity for contiguous spread and deep burrowing, the mediastinum, vertebrae, ribs, pericardium, sternum, and diaphragm may become involved. One recently reported case presented a superior vena caval syndrome along with various other signs of dissemination.[39]

Abdominal actinomycosis frequently follows an appendiceal abscess, appendectomy, or traumatic perforation of the bowel. From a localized abdominal mass characteristically situated in the ileocecal region it may extend retroperitoneally or transabdominally to produce a maze of interconnected sinus tracts, the external openings of which are most commonly in the inguinal region

and flank. More often than not the disease is recognized only after the development of persistent cutaneous fistulas or a chronic intra-abdominal abscess. However, occasionally the inflammatory component is fairly silent and the abdominal mass may be mistaken for a far-advanced visceral carcinoma.[40-42] Retroperitoneal extension may lead to involvement of vertebrae, kidneys, Fallopian tubes, ovaries, etc. In rare instances there may occur a severe pylephlebitis resulting in multiple chronic liver abscesses. Subphrenic abscesses may extend through the diaphragm to involve pleura and lung. How extensive a process may result is beautifully illustrated by a case cited by Ogilvie[43] in which the injection of Lipiodol into a sinus in the right iliac fossa outlined a tract extending upward into the liver then through the diaphragm to produce a bronchogram.

VOL.
5
1957



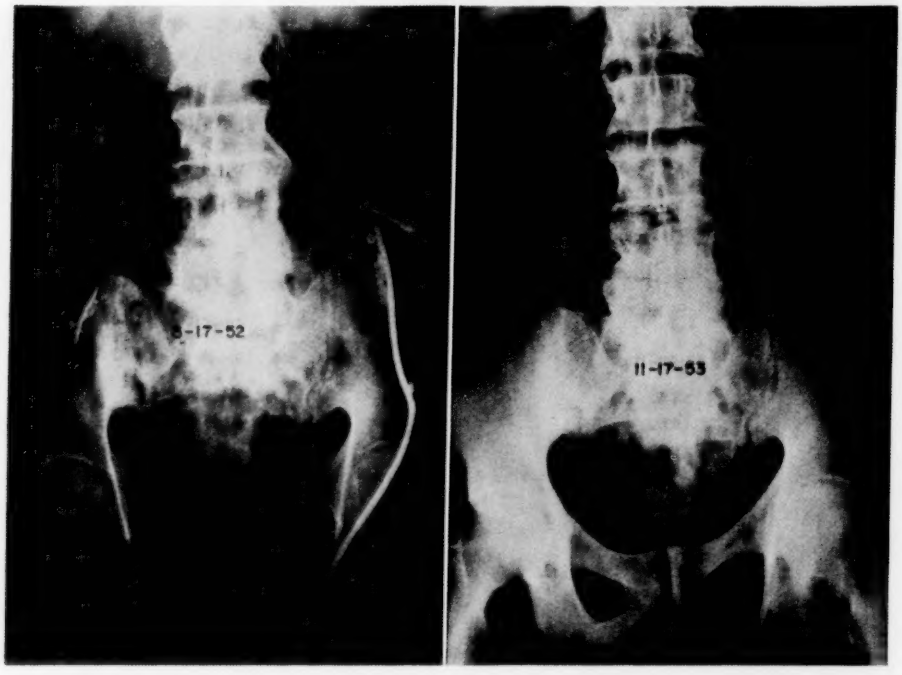
A. *B.*

Fig. 7.—Actinomycosis of mediastinum, lung, and vertebrae. Negro man with severe back pain for preceding year associated more recently with fever. *A*, X-rays showed left pulmonary density and extreme mediastinal widening most marked inferiorly. Spine films showed vertebral involvement of T_2 through T_4. Mediastinal abscess pointed posteriorly and was drained. *A. bovis* cultured from pus. On intensive penicillin therapy, augmented for several months with sulfadiazine and body cast, healing gradually ensued (*B*).

In a small percentage of cases the primary site is other than those described above. Cerebral involvement may occur, but this is far more characteristic of nocardiosis and in both diseases is felt to result from a primary pulmonary process. Likewise there exist examples of so-called primary renal actinomycosis but a more common mode of kidney involvement seems to be via retroperitoneal rather than hematogenous spread. Anorectal actinomycosis may arise either as a primary infection or secondary to a separate source within the pelvis. Skin involvement usually results from underlying sinus tracts, much less frequently from widespread hematogenous dissemination, and rarely from primary inoculation by such means as a human bite[44-50] or other wound.[51-52]

Radiographic Manifestations.—Early in the course of cervicofacial lesions there is generally no radiographic evidence of any osseous changes, but later periostitis or bone destruction may be noted in x-rays of the jaw. Occasionally the extent of the disease is appreciated only after the injection of radiopaque oil into the sinus tracts.

The radiographic findings in thoracic actinomycosis may be suggestive but are never diagnostic. It is usually indistinguishable from pulmonary tuberculosis, although an oft-quoted distinction is that actinomycosis involves chiefly



A. *B.*

Fig. 8.—Abdominal actinomycosis. Negro man who for the past 20 years had had multiple draining sinuses of the abdominal wall, lumbar and gluteal regions, and both groins. On at least four occasions he had been treated empirically for tuberculosis with streptomycin and PAS. *A*, X-ray taken after Lipiodol injection of sinus tracts shows their extent plus severe involvement of the sacroiliac area and lumbar spine. Diagnosis of actinomycosis proved by direct examination and culture of pus and biopsy of sinus tract. Treated for 6 months with penicillin, the last 4 on an outpatient basis. *B*, Sinuses closed and back pain disappeared.

the base of the lungs. A tendency toward bilateral parenchymal infiltrations is another frequently cited feature,[4] but this has not been our experience and reflects, we feel, the advanced stage in which so many cases are diagnosed. The parenchymal lesion may be small when first detected, but more typically, by the time x-rays are obtained, it presents as a relatively large area of infiltration or consolidative pneumonia with hazy margins. Quite commonly there is an associated pleuritis. Small round lucencies representing multiple abscesses may be seen within the zone of consolidation, but frank cavitation is unusual.

Occasionally one sees a dense parahilar lesion simulating bronchial neoplasm. However, for it so to resemble a carcinoma as to necessitate exploratory thoracotomy[53] (a situation often encountered in blastomycosis) is most unusual in actinomycosis.

Empyema is almost customary. There may be evidence of chest wall involvement with extensive periosteal proliferation or even destruction of ribs. The latter may be drawn together and the interspaces narrowed. Soft tissue swelling may be demonstrable. Mediastinal widening is not infrequent, but hilar lymphadenopathy is not ordinarily seen and hematogenous dissemination with miliary lung involvement is decidedly rare.[54] Any pulmonary lesion manifesting a large area of consolidation resembling lobar pneumonia or early lung abscess that remains essentially unchanged over a few weeks' time should suggest the possibility of actinomycosis or nocardiosis especially when accompanied by pleural or chest wall involvement.

VOL.
5
957

Another important difference between actinomycosis and blastomycosis is seen in the type of vertebral involvement. Blastomycosis of the spine simulates tuberculosis; actinomycosis is quite different.[55] In actinomycosis vertebral involvement is usually the result of extension from a retroperitoneal or mediastinal process. Chiefly the bodies and transverse processes are affected, and accompanying the slow process of bone destruction there is usually enough new bone formation to prevent collapse of a vertebra such as one sees so commonly in vertebral tuberculosis. Likewise the intervertebral discs are seldom involved as in tuberculosis.[56] As a result, the roentgenologic picture is rather characteristic, consisting of long dense spurs and sclerotic changes in the lateral portions of the involved vertebrae.[55] Primary vertebral disease must be quite rare but a remarkable "soap-bubble" appearance has been ascribed to it.[55]

Pathology.—The histology of actinomycosis falls somewhere between an acute suppurative and chronic granulomatous process. The more typical histologic appearance is that of a purulent infection consisting of many minute abscesses surrounded by granulation tissue. The fibroblastic reaction may be quite prominent. The cellular response consists mainly of polymorphonuclear neutrophils, but giant cells are occasionally seen, especially in the vicinity of actinomycotic granules.

These granules represent the classical histologic feature of actinomycosis and are most apt to be found within microabscesses (Fig. 4). In tissue sections stained with hematoxylin and eosin the center of the granule stains more intensely with hematoxylin and the periphery with eosin. When clubs are present around the margin, they appear eosinophilic. In periodic acid-Schiff stained tissue sections, the granules are red and the clubs or sheaths are green, indicating that the sheaths represent a tissue reaction of the host not the organism.

Treatment.—Prior to the days of effective chemotherapy the treatment of actinomycosis was discouraging. Assorted time-honored measures were used such as surgical drainage, iodides, thymol, and irradiation, but except in the case of cervicofacial lesions such treatment was seldom beneficial. Inasmuch as it is one of the more purulent of the chronic granulomatous diseases, sound

principles of surgical drainage are still indispensable in its management, but otherwise the treatment has changed radically.

The introduction of the sulfonamides and particularly of penicillin have improved the outlook considerably. By 1948, penicillin was established as the drug of choice for actinomycotic infections; Nichols and Herrell[57] reported 60 patients treated by this means. Their impression was that its effectiveness varied somewhat with the location of the lesion; over 90 per cent of those with cervicofacial, 80 per cent of those with abdominal, and 40 per cent of those with pulmonary involvement recovered on penicillin alone. Continued use of penicillin began to be associated with more and more failures due primarily to inadequate initial dosage. In one such case[58] streptomycin apparently arrested the disease after penicillin, sulfonamides, iodides, and surgical measures had failed. Garrod[59] subsequently published in vitro sensitivity tests showing that all 3 of the original broad-spectrum antibiotics (chlortetracycline, chloramphenicol, and oxytetracycline) should be effective, certainly to a greater degree than streptomycin although not necessarily as promising as penicillin. This proved precisely true and there are now well substantiated cures from chlortetracycline,[60-64] chloramphenicol,[65] and oxytetracycline.[66] In addition a most remarkable arrest of a widely disseminated case has recently been achieved with tetracycline.[39] Several cures with isoniazid[67,68] and one with stilbamidine[69] have also been reported.

Penicillin remains the drug of choice but must be given in moderately high dosage for prolonged periods of time if a relapse is to be prevented. One to five million units should be given daily for a minimum of 6 to 8 weeks. Even so an occasional failure will attend the treatment of systemic actinomycosis with penicillin alone and for that reason some consider it advisable to give another drug along with penicillin. Sulfadiazine has long been used in this manner, but in recent years there has been a swing toward the broad-spectrum antibiotics because of their greater effectiveness by in vitro sensitivity tests. The latter are not practicable for the ordinary laboratory, however. Moreover, the prolonged administration of broad-spectrum antibiotics is undesirable and should be used only if the patient has been sensitized to penicillin or if he fails to heal after adequate penicillin therapy. Even then, before blaming penicillin one should make certain that some other disease is not responsible for the persistence of the infection.

Pulmonary resection is rarely necessary for the complete obliteration of the disease and its sequelae.[70]

NOCARDIOSIS

Historical Highlights.—Like actinomycosis, nocardiosis was also first described in cattle. In fact it was little more than 10 years after Bollinger's original description of bovine actinomycosis,[6] and several years before Wolff and Israël[10] were finally successful in culturing *A. bovis* from a patient with human actinomycosis, that Nocard[71] in 1888, isolated an aerobic acid-fast actinomycete from cases of bovine farcy, a glandular disease of cattle previously attributed to tuberculosis. This organism was later designated *Nocardia farcinica*. The first

proved case of nocardiosis in the human was reported in 1890, by Eppinger[77] who proposed calling the organism *Cladothrix asteroides* because of its branching and star-shaped colonial appearance. Subsequently this fungus became better known as *Streptothrix eppingeri* and *Actinomyces asteroides*, but the term *Nocardia asteroides* as suggested by Blanchard[73] in 1896, ultimately gained taxonomic preference.

Additional case reports appeared so sporadically that more than 30 years after Eppinger's original report Henrici and Gardner,[74] in an elaborate review of the world literature (1921), could find only 26 cases of nocardiosis and the authenticity of some of these, like their own case report, does not stand up under close scrutiny.[75] From that time on there was a general tendency to regard nocardial infections, meaning primarily those due to *Nocardia asteriodes*, as exceedingly rare, highly fatal and at best no more than exotic variants of actinomycosis. In fact, until recently this particular mycotic infection was virtually unknown to most clinicians. Now, as a result of the current upsurge of interest in the subject, nocardiosis is recognized as a separate and distinct disease, probably more common than is generally believed and amenable to therapy if instituted early. Although there are several species of pathogenic Nocardia, this discussion will be limited to those caused by *N. asteroides*.

OL.
5
957

Epidemiology.—Nocardiosis is a subacute or chronic suppurative disease affecting primarily the lungs and showing a predilection for systemic spread, especially to the central nervous system where it resembles a brain tumor, brain abscess, or meningitis, and to the subcutaneous tissue where it tends to simulate actinomycosis. The few authentic cases that have been reported, and they number only slightly over 50, exhibit a rather even geographic distribution from all parts of the world. Because Nocardia (unlike the anaerobic Actinomyces) are recoverable from soil,[76-78] nocardiosis is considered exogenous rather than endogenous in origin. There is no racial preference. Any age group may be affected, the average patient being 35 to 40 years old. Men are infected more than twice as frequently as women. Although nocardiosis was first recognized in cattle[71] and despite increasingly frequent reports of canine nocardiosis,[79-81] and a recent outbreak of nocardiosis in kangaroos,[82] the disease is apparently uncommon among animals. Neither man to man nor animal to man transmission has ever been recorded.

Pathogenesis.—The preponderance of pulmonary infections points to the respiratory tract as the usual portal of entry. However, as if to emphasize its basic relationship to actinomycosis, nocardial infections have also been known to follow tooth extraction,[83-85] trauma,[52,86] and appendiceal perforation.[87] While a cervicofacial form of nocardiosis is rarely seen, the genus Nocardia is one of the groups of organisms responsible for localized infections involving the foot and ankle (mycetoma).[88-92]

Mycology.—Many different species of Nocardia have been described; fortunately few have been associated with human disease. Certainly the most important member of the genus, and the only one with which this paper is concerned, is *Nocardia asteroides*, an aerobic, gram-positive, branching filamentous fungus that is variably acid-fast. It accounts for most cases of nocardiosis

and for a variety of reasons its identity is liable to be missed. Because of its pronounced tendency to fragment into rodlike segments, *N. asteroides* may pass for bacteria. Being acid-fast and prone to pulmonary involvement it may closely mimic and be confused with the tubercle bacillus. Because of its relatively rapid growth and chromogenic qualities, it may be discarded as a saprophytic mycobacterium. Even if recognized as a fungus it is apt to be accepted as *Actinomyces bovis* despite its aerobic growth, an error augmented by the ability of *N. asteroides* to produce yellowish granules in exudate identical with those of *A. bovis*.

Minor cultural variations have been noted between strains of *N. asteroides*, but such distinctions afford no practical advantage. Henrici and Gardner[74] felt that the organisms recovered from previously reported cases of nocardiosis fell into 4 strains to which they added a fifth. Subsequently Henrici[93] revised his view and stated that the species *N. asteroides* varied through so wide a range that if species differentiation were to be based on small differences in biologic characteristics, there would be nearly as many species as strains of Nocardia isolated. Baldacci[94] and later Umbreit[95] proposed reducing the organisms of Eppinger,[72] Henrici, Gardner,[74] and others, along with all similarly aerobic, acid-fast actinomycetes to the category of variants of *N. asteroides* regardless of certain minor biologic differences.

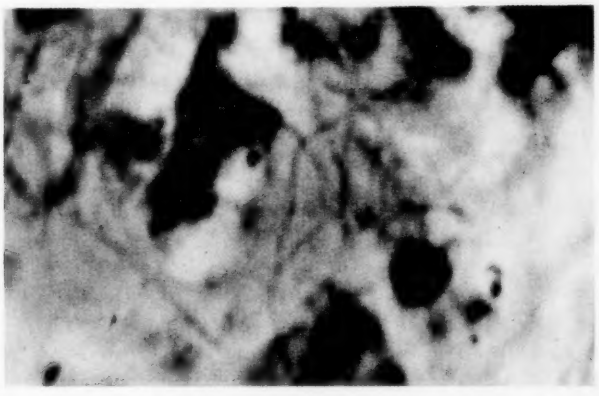


Fig. 9.—Acid-fast Nocardia in exudate. Smear of purulent drainage from a case of nocardiosis stained by the Ziehl-Neelsen technique discloses myriads of long, delicately branching, moderately acid-fast filaments. When fewer organisms are present and fragmentation into bacillary forms is more marked, Nocardia can be mistaken for tubercle bacilli, although they are generally much less acid-fast. Ziehl-Neelsen stain.

Nocardia asteroides grows well on any of the common laboratory media both at room and incubator temperatures. This applies equally well to blood agar, Sabouraud's glucose agar, and various media used for isolation of tubercle bacilli. However, the property of acid-fastness is better developed in glycerin agar or milk. Growth usually appears within 7 to 14 days. Both in colonial appearance and acid-fastness, *N. asteroides* may be quite like either the saprophytic or virulent Mycobacteria. Colonies may be creamy or pale yellow in

color and occasionally chalky white, although generally exhibiting some degree of orange, pink, red, or tan coloration. Especially on Petrik's or Petragnani's medium growth may resemble *M. tuberculosis* except for the faster appearance of the Nocardia.

Acid-fastness is characteristic of *N. asteroides*. While admittedly variable in degree, this tinctorial property has been present in all tissues and exudates that we have examined when infection was due to *N. asteroides*. Most strains completely retain the carbolfuchsin only if decolorized more briefly than usual with acid-alcohol or if destained with acid water instead. Some strains, however, particularly in pus or other exudate will be found by the ordinary Ziehl-Neelsen staining method to be completely acid-fast, resisting acid-alcolol decolorization about as well as *M. tuberculosis*. One of us (J.H.S.) has placed on demonstration Ziehl-Neelsen preparations of *N. asteroides* in sputum that could not be differentiated from companion slides of *M. tuberculosis* in sputum, even when examined by experienced personnel. Quite commonly one will encounter partial acid-fastness, fragmented portions being beaded in red and unfragmented mycelium showing red granules centrally within some filaments. Subcultured strains may lose much of their acid-fastness unless grown in milk.

OL.
5
957

It has been stated[96] and at present is widely believed that sodium hydroxide digest methods for concentration of sputum and gastric washings always kill Nocardia. This is not entirely true. Concentration procedures may prevent Nocardia from growing, especially when the specimen contains few organisms, but occasionally *Nocardia asteroides* will grow out of a concentrated specimen. In fact, of all the fungi tested by Ajello's group[97] *N. asteroides* was the one that most consistently survived concentration procedures for tubercle bacilli. In such instances the colony is apt to make a delayed appearance, and this may further confuse the picture. We have seen several occasions in which concentrated material was planted on Petrik's or Petragnani's medium and gave rise to creamy or slightly yellow colonies of acid-fast bacillary forms after 14 to 21 days of incubation at 37° C. It was assumed, therefore, that such colonies represented *M. tuberculosis*, but subculture onto Sabouraud's and Petrik's media resulted in good growth at room temperature, thus demonstrating very clearly that they were not *M. tuberculosis*. Further study showed them to be *N. asteroides*.

Final assurance that the Nocardia in question is *Nocardia asteroides* depends upon the demonstration of its pathogenicity for laboratory animals. This is admittedly a poor defining characteristic, since various fungi including *H. capsulatum* and others show considerable variation in animal pathogenicity. The same may be true of *N. asteroides*. *Nocardia asteroides* injected intraperitoneally is usually pathogenic for guinea pigs; at least to the extent of producing localized disease. If a fairly large inoculum is used, the guinea pig ordinarily succumbs within a week or two.[98] Strauss and his co-workers[99] found it necessary to add 5 per cent hog gastric mucin to the inoculum in order to produce a lethal infection in mice.

Brueck and Buddingh[100] found several strains of *N. asteroides* which, upon injection into the yolk sac of chick embryos produced death of the embryo within

4 days. Our own experience has been confirmatory and we now feel that the most satisfactory method of isolating and identifying the organism is the inoculation of the yolk sac of embryonated chicken eggs. Not only does it provide a good culture medium but helps in establishing the identity of the organism.

It must be remembered, though, that just because the isolate is pathogenic for animals is no proof that it is responsible for whatever disease the patient may present, for *Nocardia asteroides* can reside in the tracheobronchial tree in a purely saprophytic role. This is particularly true of patients with bronchiectasis and emphysema. It is often difficult, therefore, to assess the exact significance of this fungus when demonstrated only in sputum.

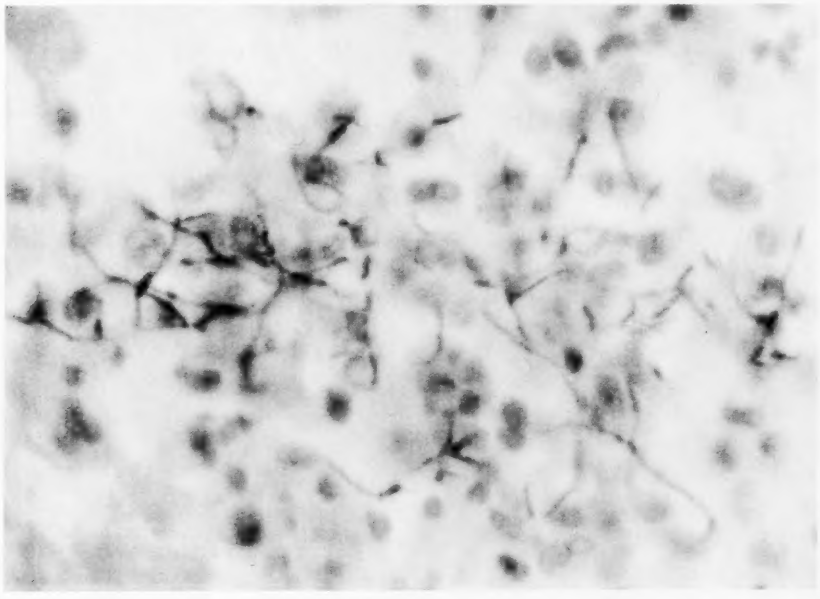


Fig. 10.—Nocardia in tissue. Tissue section of lung from fatal case of nocardiosis. Gram's stain reveals many slender, gram-positive, branching hyphae invading tissue.

When recovered from deep abscesses or closed tissue spaces such as the pleural cavity, its relationship to the pathology involved is more clearly established. Even then it is extremely important that a more significant organism not be found, because *N. asteroides* is an opportunist and it may be present solely as a secondary invader. It is on this account that the authenticity of certain reported examples of nocardiosis are unacceptable to us.[87,102] The best proof is the visualization of gram-positive hyphae invading tissue plus an associated suppurative or granulomatous tissue response (Fig. 10). Because of its relatively weak antigenicity there is no reliable skin test nor serologic aid.

Clinical Spectrum.—Certainly the outstanding clinical distinction between nocardiosis and actinomycosis is the propensity for hematogenous spread in nocardiosis due to *N. asteroides* in contrast to the contiguous spread that typifies

VOL.
5
1957

A.

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Fig. 11.—Nocardiosis proved at autopsy. Forty-nine-year-old Negro truck driver with cough and 50 pounds weight loss over the past year. Acute symptoms for preceding 2 weeks included purulent bloodstreaked sputum, right chest pain and fever. Chest x-rays showed homogenous consolidation of the right upper lobe extending across the minor fissure to involve the margin of the middle lobe (*A*). Patient was almost moribund from the start and died within 3 weeks despite antituberculous chemotherapy. An autopsy revealed nocardiosis of the lungs (*B*), brain, and kidneys with *N. asteroides* being recovered from all involved organs and demonstrated in tissue sections.

actinomycosis. This is not to say that nocardiosis cannot mimic to perfection all the manifestations of actinomycosis, for it does indeed produce chronically draining sinuses and pleuropulmonary disease indistinguishable from those caused by *A. bovis*. The difference lies in the far greater tendency for Nocardia infections to disseminate. Thus, from a primary nocardial focus in the lungs almost any organ may become involved with subcutaneous and central nervous tissue standing out as the most common secondary sites.

In about 75 per cent of *Nocardia asteroides* infections a pulmonary component is quite conspicuous with the presenting complaints in many instances being referable to the lungs. A productive cough is usually noted. The sputum is characteristically purulent and not infrequently bloodstreaked. Pleural involvement often ensues and may make itself known by the advent of pleuritic or dull aching chest pain. However, just as in tuberculosis (the disease to which it is invariably likened) there may be very little in the early stages to indicate an active pulmonary process. In fact, even an extensive nocardial pneumonitis

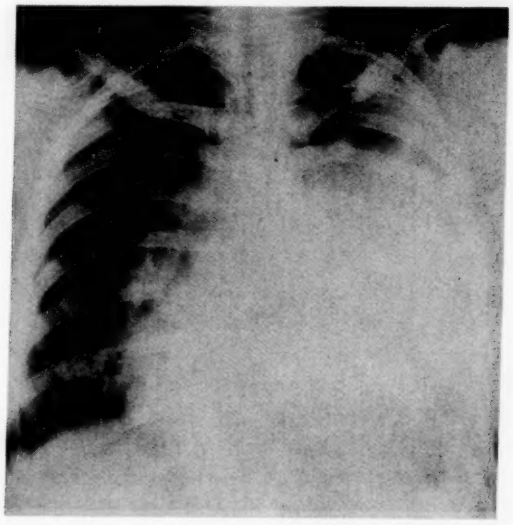

Fig. 12.—Nocardiosis diagnosed terminally. Thirty-eight-year-old housewife who had had a "chest cold" for almost a year before being admitted to the hospital with almost complete consolidation of the left lung. *N. asteroides* was isolated from sputum but patient died soon after institution of sulfadiazine therapy. At autopsy there was nocardial involvement of the lungs, kidneys, and adrenal glands with *N. asteroides* recovered in pure culture and demonstrated in tissue.

may be overshadowed by striking cerebral manifestations or by a severe constitutional response in the form of fever, night sweats, weight loss, and malaise. The onset of the disease is occasionally explosive and rapidly progressive, but contrary to frequent expressions in the literature, nocardiosis is as a rule not acute, although the tendency for sudden intensification of chronic, low-grade symptoms or the abrupt onset of dissemination will sometimes make it appear so.

VOL.
5
1957

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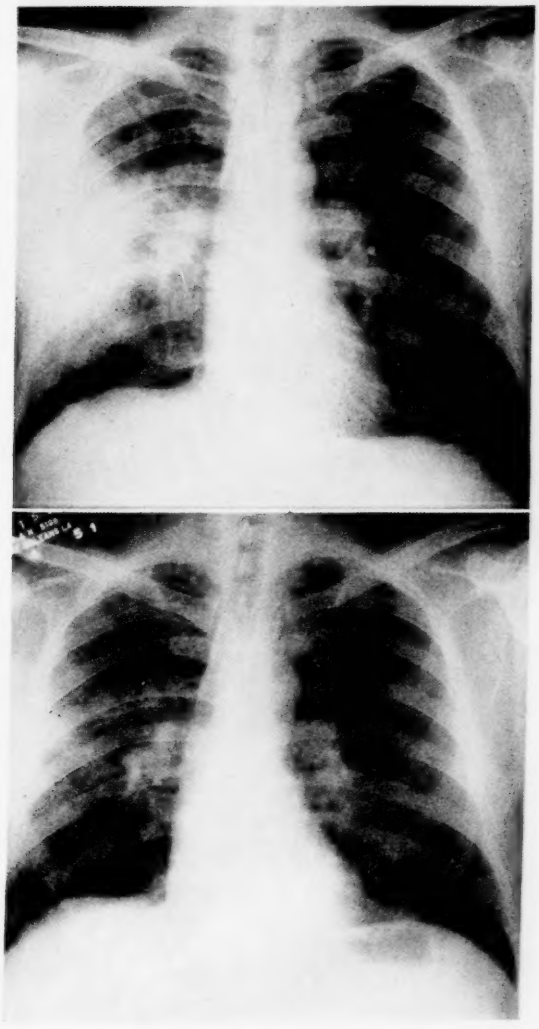

B.

Fig. 13.—Nocardiosis proved and apparently cured. Thirty-nine-year-old electrician with cough and right pleuritic chest pain of about one year's duration. *A*, Chest roentgenograms revealed a dense infiltration involving the right midlung field. Candida and Aspergillus were isolated from sputum. An abscess later appeared on the right shoulder and atypical acid-fast bacilli were seen in smears of aspirated pus. *N. asteroides* was obtained in pure culture. *B*, Apparently cured after 9 months' treatment with sulfadiazine, the drug of choice in this disease.

As in actinomycosis, empyema is a common complication of pulmonary nocardiosis occurring in about one-fourth of all cases. Many of these go on to form chest wall fistulas by a process of direct extension (empyema necessitans), but in contrast to actinomycosis nocardial fistulas more frequently result from subcutaneous abscesses secondary to hematogenous dissemination, the abscesses subsequently rupturing onto the skin to produce chronic sinus tracts. By grouping both types of fistula together it can be said that over one-third of all patients with nocardiosis will eventually present one or more cutaneous sinuses and it is not rare for this to constitute the initial complaint.

Brain involvement has always been a prominent feature in various descriptions of nocardiosis beginning with Eppinger's classic report of a patient with pulmonary "pseudotuberculosis," brain abscess, and meningitis.[72] The relative incidence of cerebral spread is comparable to that of cutaneous fistulas, about 30 per cent of Nocardia infections involving either the brain or meninges. Depending upon whether there is appreciable fever, the cerebral signs will be interpreted as a brain abscess or tumor, and in this regard it is important to note that in a significant proportion of such cases fever is absent[84,103-107] or unimpressive.[108-111] Despite the presence of coexisting pulmonary disease the presenting sign may be cerebral,[108,112-116] and in a fair number of cases there has been no definite evidence of nocardiosis outside of the central nervous system.[84,105,107,108,110,111,117] This occasional lack of demonstrable pulmonary disease in the face of cerebral nocardiosis deserves comment, since it is a situation analogous to that seen in cryptococcosis. In both diseases there is an affinity for cerebrospinal involvement which is in turn a common cause of death. Likewise in both diseases the primary pulmonary focus may be difficult if not impossible to demonstrate, having gone on to complete resolution while a secondary cerebral or meningeal lesion has caused the patient's demise. Ordinarily, however, either an antecedent or coexisting pulmonary lesion is recognized, thus suggesting the possibility of bronchogenic carcinoma or tuberculous meningitis. In any event the incidence of diagnostic craniotomy in these patients is very high.

In the course of dissemination almost any other organ may be involved, particularly the kidney[108,118-124] but also on rare occasions the adrenal glands, pancreas, etc. Nocardial peritonitis is another uncommon occurrence. As mentioned previously some species of Nocardia are a cause of mycetoma, a chronic granulomatous lesion of the extremities especially the feet, although they are less important causal organisms than the ascomycetes.

Having ourselves had one patient with coexisting nocardiosis and tuberculosis, it is interesting to note other reports of these two diseases in combination.[110,125] We cannot agree with McQuown,[126] however, that as many as 5 per cent of all hospitalized patients with tuberculosis have nocardiosis. Nocardiosis has also been reported in association with leukemia,[123,125] Hodgkin's disease,[127] and diabetes.[108]

Radiographic Manifestations.—The pulmonary pattern varies from little or no visible involvement to extensive bilateral pneumonitis. Occasionally the parenchymal lesion will be limited to a relatively small streaky infiltrate but more

characteristically it is represented by a dense area of lobular or lobar consolidation. An entire lung field may be obliterated in this fashion. The consolidated area may contain multiple round zones of rarefaction or large radiolucent areas suggesting lung abscess. Cavitation occurs more often than with actinomycosis but is not common. Associated pleural reaction is typical and there is often evidence of empyema. The parenchymal component will rarely be round enough to suggest a circumscribed tumor mass[125] and, like actinomycosis, nocardiosis has never been incriminated as a cause of a so-called tuberculoma. Nevertheless the development of cerebral signs in a patient being followed for a pulmonary lesion is likely to support a diagnosis of bronchogenic carcinoma. Roentgenologic evidence of lymph node enlargement is unusual, although bronchopulmonary nodal involvement has been encountered at autopsy and Nocardia have been recovered from resected mediastinal lymph nodes.[128]

OL.
5
957

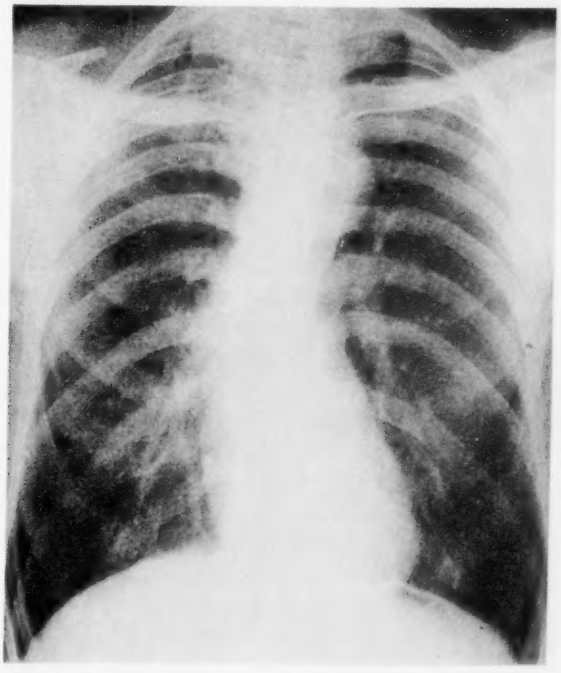

Fig. 14.—Emphysema—Nocardia in Sputum. Sixty-two-year-old Negro who was hospitalized for severe pulmonary emphysema. Chest roentgenogram revealed no other pulmonary process. *N. asteroides* was repeatedly recovered from his sputum. Despite the fact that it was strongly pathogenic for laboratory animals, the Nocardia in this instance was considered a tracheobronchial saprophyte.

Terminally there is a proclivity for extensive bilateral spread, but although miliary involvement has been described at autopsy only one case has been recorded in which x-rays showed the development of diffuse micronodular densities comparable to those of miliary tuberculosis.[124]

Pathology.—The histology of nocardiosis is usually more suppurative than granulomatous, most lesions amounting to frank abscesses. There is seldom

as marked a fibrous reaction as one encounters in actinomycosis, and so-called sulfur granules are infrequently seen in tissue. Grossly there may be a striking resemblance to tuberculosis, but the presence of Langhans type multinucleated giant cells is an exceptional finding[111,118,123,129] as is caseation necrosis. The case of Connar and co-workers[130] is noteworthy in that the reaction in resected pulmonary tissue was originally misinterpreted as lipoid pneumonitis. The typical picture is one of multiple abscesses in which the cellular response consists mainly of polymorphonuclear leukocytes.

Nocardia are not detectable in tissue by ordinary staining methods such as hematoxylin and eosin. The standard Ziehl-Neelsen technique may reveal branching filaments exhibiting weak to moderately strong acid-fastness. The filaments also take the periodic acid-Schiff stain but are difficult to distinguish from reticulum, which also presents a Schiff-positive, branching, filamentous appearance. The best staining method for the demonstration of actinomycetes in tissue is the Gram stain which beautifully delineates the hyphae as slender, gram-positive, beaded, and branching filaments measuring approximately one micron in diameter. Of course, for proper identification of the organism, its recovery in culture is essential.

Treatment.—The treatment of nocardiosis is notoriously ineffectual, largely because the diagnosis is so often made late, if indeed it is established ante mortem. However, there have been a small number of cases of systemic nocardiosis due to *N. asteroides* which appear to have been cured,[85,96,107,111,117,130-133] and to these we can add four cases of our own. We have purposefully omitted reference to other cured patients in whom the identity of the causal organism is open to question,[104,106,134] or the possibility of its being a secondary infection has not been resolved.[87,102,122]

Because of their early success in treating actinomycosis with the sulfonamides, the Duke group[96] used these drugs in several cases of nocardiosis, and in two a successful result was obtained. These are the first recorded cures of systemic nocardiosis (1944). In 1946, Kirby and McNaught[108] reported supporting proof of the effectiveness of sulfadiazine in a patient with pulmonary nocardiosis who responded repeatedly to short courses (probably inadequately prolonged) of this drug only to develop disseminated disease from which he ultimately died. The patient reported by Shaw, Holt, and Ray[131] was cured after intensive penicillin therapy but the most marked improvement followed the addition of sulfadiazine to the therapeutic regimen. The Mayo Clinic group[132] reported their first cure in 1948 and emphasized the need for intensive and prolonged administration of sulfadiazine, their patient requiring a total of 1,268 Gm. over a 5-month period. This patient, a physician, continues to do well and has shown no evidence of reactivation.[125] Also in 1948 Jacobson and Cloward[117] recorded a case of severe nocardial meningitis cured by combined sulfadiazine and penicillin therapy. A patient with *Nocardia asteroides* cervical adenitis secondary to a tooth extraction was apparently cured by extended sulfadiazine administration along with potassium iodide.[83] In 1951, a third successfully treated case was reported from Duke University,[130] once again illustrating the need for vigorous sulfonamide therapy in conjunction with surgical drainage. Cerebral abscesses

due to *N. asteroides*[107,111] have been cured apparently by craniotomy, on one occasion with[111] and on one without[107] ancillary sulfonamide administration. The recent case of Thomas and Pulaski[85] is representative in that improvement ensued, not after tetracycline and erythromycin, but only after sulfadiazine was begun. Thus there is ample testimony that sulfadiazine is the drug of choice. Nevertheless, *Nocardia asteriodes* is relatively resistant, and for maximum effectiveness it is probably advisable to administer concurrently some other drug such as one of the broad-spectrum antibiotics or streptomycin, any one of which usually exhibits a mild inhibitory effect.

In choosing the best chemotherapeutic combination one must be aware that sensitivity tests can be deceptive. Several workers[99,135] have demonstrated, for example, that drugs like chlortetracycline and streptomycin may exert a greater suppressive effect in vitro against *N. asteroides* than does sulfadiazine, yet be decidedly inferior to sulfadiazine in vivo. In animal protection experiments against nocardiosis, Strauss and co-workers[99] showed sodium sulfadiazine to provide almost perfect protection. Halde and Newstrand[136] found sulfadiazine to be relatively ineffective in vitro against most strains of *N. asteroides* but the same drug in fairly large dosage significantly lowered the death rate in mice infected with one of the nonsensitive strains. From this they concluded that fungistatic action at concentrations below that required for complete inhibition of growth is all that is needed to enable the host's natural defense mechanisms to overcome the infection.

In our experience fresh isolates of *N. asteroides* are usually significantly inhibited by sulfadiazine in a concentration of 10 mg. per cent, but the degree of inhibition is not augmented by increasing the concentration four- or fivefold. Some strains are similarly suppressed by streptomycin without any increase in inhibitory effect despite a considerable increase in the concentration of streptomycin. One of us (J.H.S.) has observed the same phenomenon with broad-spectrum antibiotics. Stilbamidine and 2-hydroxystilbamidine may be nocardiostatic to the point of producing complete inhibition by concentrations attainable in man.

In an experience with 15 cases of systemic nocardiosis we have had only limited success. However, in five instances the diagnosis was made post mortem and in several others terminally. Four patients have been cured and another was apparently cured following prolonged drug therapy and pneumonectomy but unfortunately the latter patient died postoperatively of a pulmonary embolus. Our few successes lead us to believe that the best chance for recovery rests in vigorous chemotherapy combined, when indicated, with the intelligent use of surgical drainage or resection. The combination of sulfadiazine plus streptomycin or one of the broad-spectrum antibiotics is probably a wise choice initially. A daily dose of 4 to 6 Gm. of sulfadiazine and 2 Gm. of streptomycin is a reasonable regimen for an adult, but may deserve being increased to 10 or even 12 Gm. of sulfadiazine per day in very severely ill patients. After sensitivity tests have been reported, one may choose to modify therapy, particularly when the initial response is disappointing. In this connection it must be remembered that nocardiosis is one of the chronic granulomatous diseases,

and dramatic amelioration of symptoms or prompt improvement in the x-ray picture is not to be expected.

The question of duration of therapy is comparable to that in tuberculosis and actinomycosis. Treatment should certainly be continued for several months after clinical manifestations have disappeared or stabilized, if recurrence is to be prevented. Whenever possible, therapy should be guided by interval mycologic study. One of our patients required 3 years of continuous therapy before all sinus tracts closed and permanent cure was effected.

As with tuberculosis and many of the other mycoses, surgery may be necessary to remove nocardial residua and is, of course, indispensable for the evacuation of abscesses and empyemas.

SUMMARY

As a reflection of the close relationship between their causative organisms, actinomycosis and nocardiosis present considerable over-all similarity, although possessing too many basic differences to be regarded as other than two separate and distinct diseases. Both are caused by branching, fragmenting, filamentous fungi, *Actinomyces bovis* being fastidious and growing only at incubator temperature under anaerobic or microaerophilic conditions, while *Nocardia asteroides* flourishes on all common laboratory media at both room and incubator temperatures. Both organisms are gram-positive, but *N. asteroides* is acid-fast whereas *A. bovis* is not. *A. bovis* exhibits little pathogenicity for laboratory animals; *N. asteroides* is typically quite lethal, yet either can reside as a saprophyte within the human tracheobronchial tree and elsewhere.

The natural habitat of *A. bovis* is the human mouth and oropharynx, while that of *N. asteroides* is the soil. Hence actinomycosis is considered endogenous, and nocardiosis exogenous, in origin. Actinomycosis is fairly common; nocardiosis, perhaps unjustly so, is looked upon as rare. Each has a world-wide distribution.

Clinically the two diseases may be indistinguishable, with chronic pleuropulmonary lesions, subcutaneous abscesses, and multiple draining sinuses typifying both. Any organ may be involved, but hematogenous dissemination is not only more common but almost characteristic of nocardiosis, with the combination of lung and brain involvement being quite outstanding.

Histologically there is nothing to separate the two diseases, although actinomycotic granules (sulfur granules) are more characteristic of actinomycosis than nocardiosis. In each disease the basic lesion is more suppurative than granulomatous. Because of the tendency of both organisms to fragment into bacillary forms either may be mistaken for bacteria, with further confusion resulting from the acid-fastness of *N. asteroides*.

Perhaps the sharpest practical distinction between the two diseases is in the matter of therapy. *A. bovis* is quite sensitive to penicillin, which is the drug of choice in actinomycosis, and to a lesser extent also to sulfadiazine and the broad-spectum antibiotics. *N. asteroides*, on the other hand, is resistant to penicillin, and the drug of choice is sulfadiazine. In view of the extreme seriousness of most nocardial infections sulfadiazine is probably best given in

VOL 5 195

combination with streptomycin or whatever broad-spectrum antibiotic appears most effective in vitro. In both diseases, but especially in nocardiosis, treatment must be instituted early if cure is to be effected and must be continued in high dosage for prolonged periods if relapse is to be prevented.

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VOL 5 195

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VOL. 5 1957

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OL.
5
957

NORTH AMERICAN BLASTOMYCOSIS

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NORTH American blastomycosis (Gilchrist's disease) is a granulomatous, infectious disease caused by the fungus, *Blastomyces dermatitidis* (Gilchrist and Stokes, 1898). The malady, in its cutaneous form, was first described by Gilchrist[1] in 1894. A few years later, together with Stokes, he was able to isolate and culture the causative organism which he designated as *B. dermatitibis*.[2,3] The first description of the disease in its systemic form was made by Walker and Montgomery[4] in 1902.

The first reports by Gilchrist were followed by an era of confusion during which the disease was confounded with other entities, particularly cryptococcosis and candidiasis, all caused by morphologically similar budding organisms. Nineteen new names were suggested for the causative fungus. During the last two decades, however, a clearer picture of the disease process has emerged, particularly as a result of studies by the Duke Medical School group, headed by Smith, Martin, and Conant. Numerous clinical and laboratory reports have contributed significantly to a fuller understanding of the disease, but there are still some fundamental questions to be answered. Excellent review articles on North American blastomycosis are available.[5-18]

GEOGRAPHICAL DISTRIBUTION

Martin and Smith[9] found in 1939 that 98 per cent of 340 proved cases of blastomycosis had the origin of their disease within the United States. Of the two cases originating elsewhere, one was from Canada[19] and the other from England.[20] Presumptive cases were reported from the Philippines, Egypt, and Russia, and inadequately described cases from Canada, Puerto Rico, Costa Rica, Russia, England, China, South America, France, and Mexico.

The majority of patients who develop blastomycosis in the United States come from the Southeastern and the Midwestern states. Sporadic cases have been reported from all states. Schwarz and Goldman,[21] in a recent questionnaire survey sent to 1,569 dermatologists and 403 chest surgeons throughout the United States, had reported to them 101 cases of North American blastomycosis diagnosed and/or treated during the first 6 months of 1953. They considered the disease to be common throughout the United States. The presence of blastomycosis in Canada has been confirmed beyond doubt by a number of relatively

recent reports.[11,22-26] On the other hand, acceptable evidence for its indigenous occurrence in Mexico is still lacking. Martinez-Baez[27] proved an infection by *B. dermatitidis* in a Mexican farmer with demonstration of the organism. His claim that the infection in this patient was native to Mexico, however, was made less tenable by the fact that the patient worked as a migratory worker for 8 months in California 4 years prior to the onset of clinical symptoms.

Case reports from countries outside the North American continent appear from time to time in the literature. With an increasing general interest in and knowledge of medical mycology, more physicians are aware of the possibility of the occurrence of North American blastomycosis in their own countries. Proved or presumptively proved cases have recently been reported from India,[28,29] Spain,[30] Germany,[31] Hungary,[32] Tunisia,[33,34] Venezuela,[35] Italy,[36] and Australia.[37] Modern facilities for travel, affording greater opportunities for individuals from endemic areas to develop disease in other parts of the world, complicate the situation, as exemplified by case reports of North American blastomycosis among U. S. Armed Forces personnel at foreign stations in France,[38] Okinawa,[39] and and Japan.[40]

OL.
5
957

EPIDEMIOLOGY

The precise mode of infection is not yet fully known. It is generally presumed to be exogenous, with the infective saprophytic form of the organism existing in nature. Unlike *Coccidioides immitis*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Sporotrichum schenckii*, *Blastomyces dermatitidis* has not been indubitably isolated from nature. Stober[6] claimed the isolation of an organism similar to *B. dermatitidis* from a rotting wooden plank in the home of one of his patients but this has not been confirmed. Nonetheless, the soil is strongly suspected as the natural reservoir of the organism. Emmons[41] successfully cultured *B. dermatitidis* in sterilized soil in the laboratory.

The occurrence of natural infection in dogs[42-45] and horses[46] suggests the existence of animal reservoirs. The organism has not as yet been isolated from wild animals. With the latest report of Newberne, Neal, and Heath,[45] a total of 30 cases of canine North American blastomycosis have been described in the literature. Apparently the disease pattern in dogs is similar to that in man. Pulmonary and cutaneous involvement were the most salient findings. The sole report of infection in a horse dealt with involvement of the udder of a mare proved by histopathologic sections.

The manner of transmission of the disease has remained a provocative problem. The infectivity of *B. dermatitidis* (Figs. 1 and 2) has been proved beyond any reasonable doubt by successful animal inoculation with either the mycelial or yeast phase of the organism.[47-49] Lesions on the chorioallantoic membrane of the chick embryo have been produced with the yeast phase.[50] Open skin lesions and body secretions or exudates, such as sputum and pus that gain access to the outside, teem with the tissue forms of the organism and provide excellent opportunities for contagion. Yet no direct natural transmission from man to man has been acceptably proved. A few, isolated cases of accidental mechanical inoculation through a break in the skin have been reported in laboratory

Fig. 1.

Fig. 2.

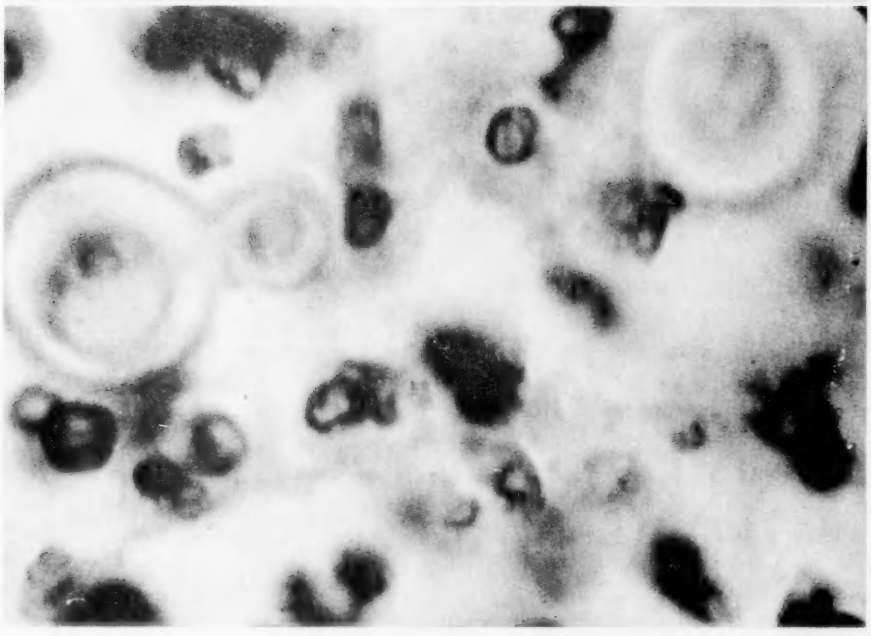

Fig. 1.—*Blastomyces dematitidis*, yeast phase on blood agar, incubated at 37° C. showing single budding of the yeast cells or organisms shortly after budding has occurred. Occasionally single abortive hyphae may be seen extending from the yeast cells in a tubelike manner. (×450; reduced ¼.)

Fig. 2.—*Blastomyces dermatitidis* in tissue showing budding, double-refractive cellular walls and granulomatous infiltrate. The yeast cells are similar to Fig. 1 or to those seen here when a direct mount is made from pus, from the border of a granulomatous lesion or from an abscess, except for the abundance of associated polymorphonuclear cells. (×1,500; reduced ¼.)

Fig. 3.

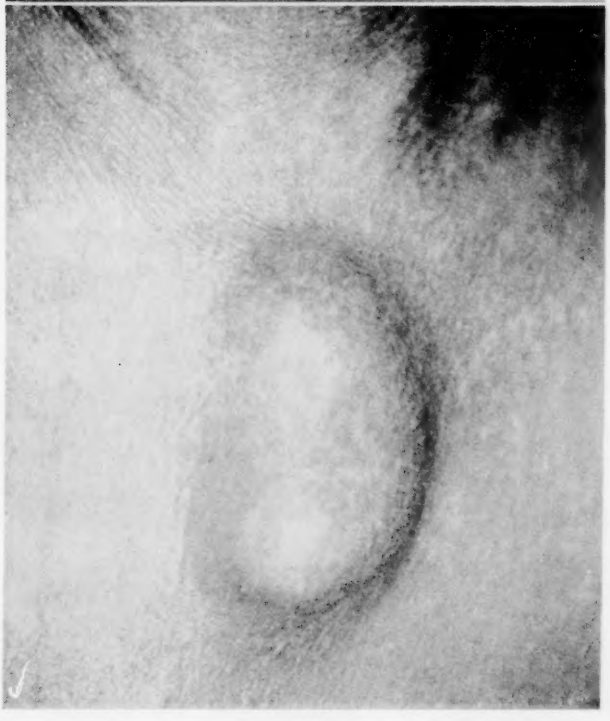

Fig. 4.

Fig. 3.—A typical granulomatous lesion of the skin due to North American blastomycosis. The border is raised, verrucuous, and sharply limited. Tiny abscesses make up this border and pressure on them with a glass slide emits pus which allows direct examination and early diagnosis by identifying the single budding organisms. The center shows various degrees of scarring depending on the duration of the lesion.

Fig. 4.—An abscess due to North American blastomycosis arising from the bony sternum and following a blastomycotic pneumonia by some weeks. Later other lesions appeared of the granulomatous as well as the abscess type.

VOL.
5
1957

workers and pathologists[13,51,52] handling cultures or infected clinical material. Schwarz and Baum[53] did not encounter a Blastomycin skin test reactor among 58 hospital workers and 48 home contacts in close association with patients having active disease. He opined that *B. dermatitidis* possesses a low index of contagiousness.

The skin and lungs are looked upon as the most probable entry of the infection. Appearance of initial skin lesions (Fig. 3) have been preceded by skin injury from thistles, thorns, slivers, nails, cinders, clams, and dog bites. This antecedent trauma has been regarded as a predisposing factor. As a result of their review of 60 cases, Schwarz and Baum[13,54] proposed a revolutionary concept to which we also subscribe: that practically all cases of North American blastomycosis are pulmonary in origin, including most cases hitherto regarded as primarily cutaneous.(Fig 4.) Consequent to this thesis that the disease is essentially a pulmonary infection, a parallelism between the disease pattern of North

Fig. 5.—Typical North American blastomycosis with scarring, atrophy, activity, and healing. The "centrifugal character of growth" of the granulomatous lesions is well illustrated.

American blastomycosis and those of coccidioidomycosis and histoplasmosis was drawn.[55,56] Studies on the two latter mycoses have proved the occurrence of two clinical forms—the frequent, benign, primary type and the rare, generalized, fatal form (Figs. 5 and 6). Primary infection is usually by inhalation of infective spores. If the evolution of the epidemiologic knowledge on North American blastomycosis were to follow that of coccidioidomycosis and histoplasmosis, it is essentially still at the stage where only the rare, fatal, disseminated disease

is known for certain. Coccidioidomycosis and histoplasmosis were recongized in this form long before the discoveries of the mild primary infections. In an attempt to demonstrate the existence of a prevalent benign North American blastomycosis, Furcolow and associates[55] conducted a skin test survey with tuberculin, Histoplasmin, and Blastomycin among 7,194 school children in Hamilton County, Ohio. No supporting evidence was obtained. The 12 per cent positive reactions to Blastomycin were regarded as cross reactions to Histoplasmin. Only one child was a Blastomycin reactor with a negative Histoplasmin reaction. The possibility of the occurrence of widespread, mild infections cannot be dismissed on the strength of negative results of skin test surveys alone. Subclinical and spontaneously healing cases of North American blastomycosis have been reported.[15,17,57] A more significant development is a report of the appearance of the disease in an epidemic form. Bonoff[39] reported 23 cases of acute pulmonary blastomycosis among the U. S. Army personnel in Okinawa,

VOL.
5
1957

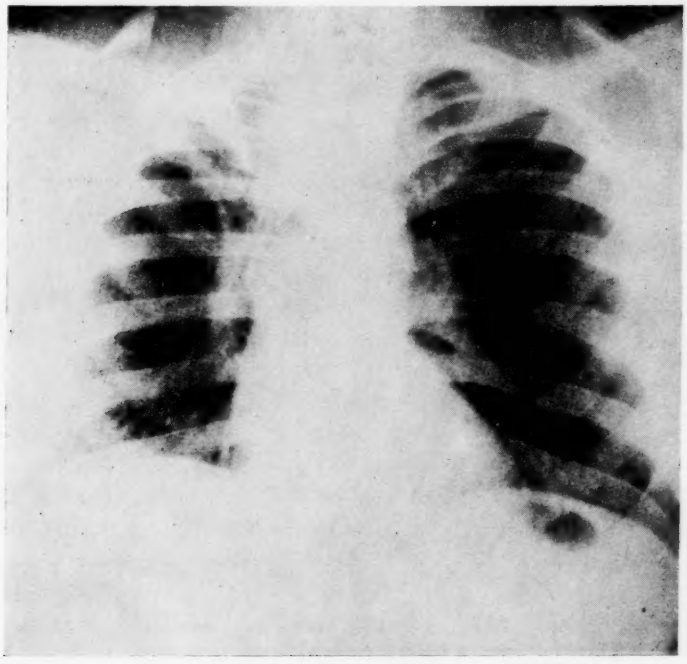

Fig. 6.—Chest of A. W. (Table I) showing both right and left upper lobe involvement with North American blastomycosis. His chest cleared with some scarring after 3.0 Gm. of stilbamidine methionate and he has remained free of the disease since 4 years and 8 months. He is now alleged to have far-advanced tuberculosis of the lungs. (From Curtis and Harrell, A. M. A. Arch. Dermat. & Syph. **66**:676, 1952.)

but unfortunately, his claims of demonstration of *B. dermatitidis* in the sputum and urine were not authenticated by cultures. A verified epidemic was observed by Smith and his associates[57] in Grifton, Pitt County, North Carolina. The sudden appearance of 10 pulmonary cases in the locality provided an excellent opportunity for epidemiologic investigations. A survey of the town population revealed a number of individuals with positive Blastomycin and negative Histoplasmin skin tests. The source of infection, however, was not traced.

CLINICAL FEATURES

Age.—The infection may appear in any age group. The youngest recorded patient with proved North American blastomycosis was 5 months old.[57] Kunkel and his colleagues[15] and Sutliff, Kyle, and Hobson[16] each had an 84-year-old patient in their own series. Most cases, however, occur between the ages of 30 and 50 years and the average age of incidence falls within the fourth decade of life.

Sex.—The disease is definitely more common in men than in women. The reported ratio of men to women ranges from 4.3:1 to 15:1. The reason for this sex distribution is presumed to be the greater exposure of men to infection by reason of their occupations and other activities.

Race.—No race is immune. The racial distributions in the different reported series of cases probably mirror the normal proportions of the races in the general population of the given locality.

Occupation.—Many authors regard North American blastomycosis as a disease of the poorer working class, who have more exposure to dust, soil, wood, and vegetation. However, no special occupational relationships can be definitely claimed for the disease.

Signs and Symptoms.—Since Gilchrist's first report and the demonstration a few years later by Walker and Montgomery of systemic involvement, North American blastomycosis has been classically divided into the localized cutaneous and the disseminated forms. The two types are divergent in clinical course, prognosis, and response to therapy and were thought to be due to different portals of the infection. The studies of Schwarz and Baum[13,54] and Wilson and his associates[52] change this older concept and align blastomycosis more closely with coccidioidomycosis.

Cutaneous Form.—The skin lesion usually first appears as an innocuous-looking papulopustule on some exposed area of the body, most frequently the face, wrists, hands, ankles, and feet (Fig. 7). The scalp, palms, soles, and mucous membranes are generally not affected. No portion of the body surface, however, may be considered absolutely exempt. The initial lesion enlarges slowly and may take months, even years, to develop into a fullblown, verrucous, ulcerative granuloma. A typical lesion is elevated and crusted. Upon removal of the crusts, surface bleeding is easily induced. The surface is wartlike with irregular papillary elevations bathed in a seropurulent discharge. The most characteristic feature is the border which is elevated, sloped abruptly into, and sharply delineated from, the adjacent normal skin, violaceous in color, and pinpointed with miliary abscesses. The lesion extends peripherally and as it increases in size, the central portions tend to heal with formation of soft, thin, atrophic scars. This central scar may still exhibit miliary abscesses which may act as foci of recurrences.

The lesions may be single or multiple. If multiple, they often are in close proximity to each other, but may be remote from one another. They are arciform in configuration, and coalesced lesions present serpiginous contours. The

usual size is from 3 to 4 inches in width, but may attain surprising dimensions up to 12 inches, involving the trunk or whole extremities. The regional lymphatics are seldom involved in this form. (Fig. 8.)

VOL.
5
1957

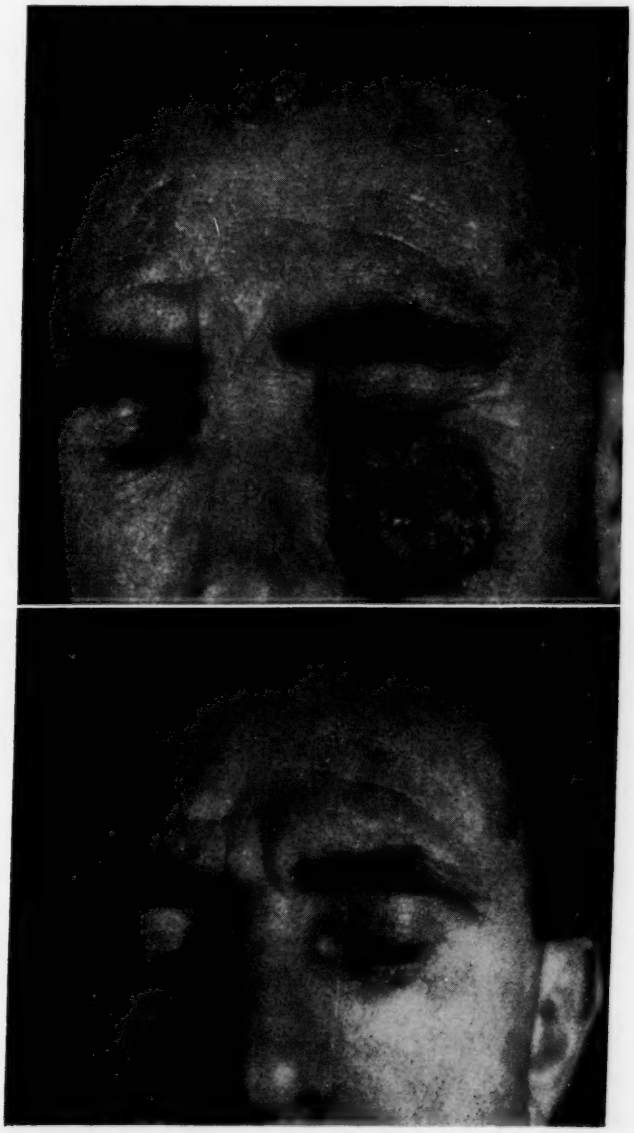

Fig. 7.—A patient with North American blastomycosis treated by curette and cautery followed by fractional roentgen ray therapy totaling 480r in air. Since most North American blastomycosis is a systemic disease, this form of therapy is rarely curative although it was in this instance.

Subjective symptoms are relatively mild, or even absent, and the general health of the patient is unimpaired except in the presence of secondary bacterial infections. The clinical course is marked by chronicity with long periods of remissions and recrudescences.

Contrary to prevailing opinion, Schwarz and Baum[13,56] considered the vast majority of the skin lesions described above as secondary to a healed or undiscovered primary focus in the lungs. This conception is gaining more proponents.[15,52] Wilson and associates[52] suggest, and we concur in the opinion, that cutaneous North American blastomycosis is a rare, but distinct clinical entity, represented so far only by 4 proved cases of definite cutaneous inoculation of

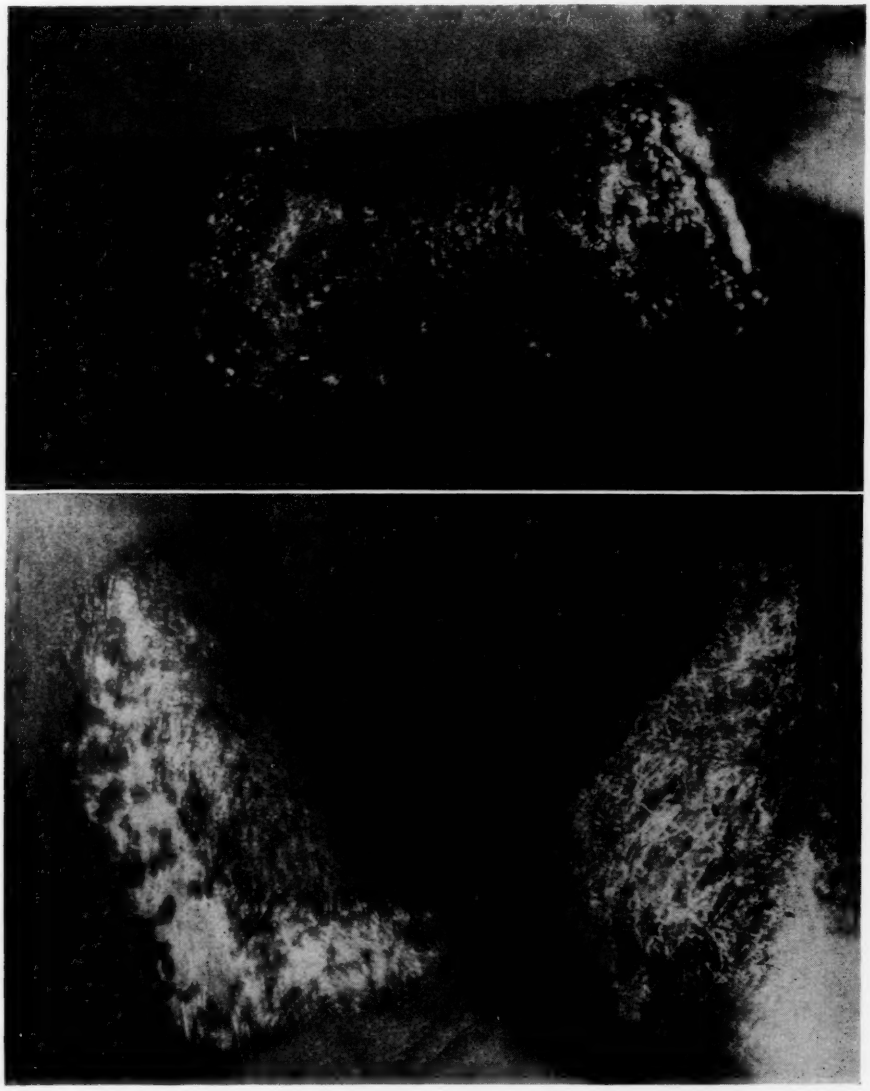

Fig. 8.—A patient with North American blastomycosis of the wrist, ankle, back, and lungs treated with 3.0 Gm. of stilbamidine (A. W., Table I) who rapidly recovered and 4 years and 8 months later had no evidence of recurrence. The wrist lesion before treatment is typical of the disease. The scarring following treatment in the second photograph is also typical. One should remember that the lungs or other organs may also scar. Neither stilbamidine nor 2-hydroxystilbamidine give a satisfactory amount of "cure" in systemic North American blastomycosis.

the organism with later chancre formation and regional lymphatic involvement. In tuberculosis, syphilis, yaws, sporotrichosis, coccidioidomycosis, and cat-scratch fever such a chancriform syndrome is expected.

Systemic Form.—This can involve any organ of the body but probably begins in the pulmonary tract most frequently. The presenting signs and symptoms depend upon the organ or organs affected and the extent of involvement. There is no clinical syndrome characteristic of the systemic disease, the clinical picture being that of any chronic infection with suppuration or fibrosis of the different organs. The tissues most frequently involved are the lungs, skin, subcutaneous tissues, and bones. The least commonly affected is the gastrointestinal tract. Many patients, especially in the advanced stages of the disease, demonstrate involvement of multiple organ systems.

The onset is frequently associated with respiratory symptoms of cough, productive sputum, hemoptysis such as may be found in other lung infections, whether bacterial, viral, or rickettsial. It usually begins insidiously, but in certain instances it may be an acute and fulminant[35,59,60] and blastomycotic pneumonia. The symptoms consist of chills, fever, productive cough, hemoptysis, chest pain, loss of weight, night sweats, and dyspnea. These may be transient and so mild as to be missed by the patient, but they may progress to dominate the clinical picture, which is so easily mistaken for pulmonary tuberculosis, carcinoma, or abscess. The infection may even assume a miliary form. The mediastinum and the pleura may be involved and the formation of sinuses through the chest wall may occur. The physical signs depend upon the nature of the lesion and are often those of consolidation with fullness, increased vocal fremitus, decreased breath sounds, and râles. Progressive cases eventually acquire the appearance of any chronic, generalized, debilitating infection with inanition, anemia, and extreme loss of weight and strength.

With progression, the disease disseminates from the pulmonary focus. Dissemination may happen even when the primary lesion shows a tendency to involute, as demonstrated by Schwarz and Baum.[13] In this case, the patient first seeks medical care for complaints referable to the organs secondarily affected via hemotogenous spread. The pulmonary focus may escape clinical recognition and be discovered only on autopsy or by a chance culture of the sputum in the face of negative x-rays.

In a number of instances, secondary involvement of the skin appears as the first sign of systemic disease. The lesions develop in unexposed parts of the body, like the trunk, as painful, deep subcutaneous nodules. These are actually abscesses with a marked tendency to break through the skin and assume the characteristics of the lesions described as primary cutaneous.

Involvement of the skeletal system is not uncommon. Martin and Smith,[9] Reeves and Pedersen,[61] Cherniss and Waisbren,[18] and Kunkel and associates[15] found the incidence of osseous and arthritic involvement in their own series 50, 47, 53, and 31 per cent, respectively. The symptoms, mainly pain and loss of function, are those of localized or diffuse osteomyelitis or periostitis and septic arthritis. In 63 cases reviewed by Jones and Martin,[62] 13 patients complained initially of arthritic pains in the back or one of the extremities. The ribs, vertebrae and skull bones, and the intervertebral knee, and elbow joints are more

commonly invaded. Invasion is attained either by direct extension or hematogenous metastasis. Blastomycosis of the vertebral axis simulates spinal tuberculosis with extensive bone destruction, marked narrowing of the disc space, vertebral body collapse, and associated paravertebral infection that may be manifested clinically as a psoas abscess.[63] In the long bones, the disease process tends to involve the epiphyses and extend directly to the adjacent joints.

Involvement of other organs is less frequently encountered. Cases affecting the genitourinary tract were reviewed by Moore and Halpern,[64] and Schwarz and Baum.[13] Symptoms listed were pyuria, hematuria, dysuria, prostatic enlargement, dribbling, and urinary retention. The female genital organs are also involved, though rarely.[65] Noojin and Praytor[66] recently recorded a patient who developed systemic blastomycosis during the second trimester of her pregnancy. A normal child was born.

Blastomycosis of the central nervous system is rather uncommon.[9,13] Depending upon the nature and location of the lesions, localizing symptoms of single or multiple abscesses in the brain or the spinal cord and manifestations of focal or diffuse meningitis are obtained.

Other seats of infection occasionally described are the eyes,[67-69] larynx,[15,71,72] gastrointestinal tract, adrenals, and thyroid.

Roentgenography.—Although sometimes a blastomycotic process may be suspected from a roentgenogram, there is no single diagnostic feature in the roentgenographic appearance of the disease. The pulmonary picture varies considerably from acute bilateral, massive, pneumonic consolidations to the small fibrotic, strandlike lesions of the quiescent stage.[11,15,18,73-76] Other lesions described are small patchy pneumonitis, lobular consolidation, mass granulomas located near the hilum and projecting into the lung fields with irregular outlines like neoplasms, "coin" lesions, lobar atelectasis, pleural thickening with or without effusion, small infrequent cavitations with thin walls and hazy outlines, and miliary shadows. Hilar and mediastinal lymphadenopathy with or without parenchymal involvement is frequent. The roentgenographic findings are usually mistakenly diagnosed as pulmonary tuberculosis or neoplasm. The other pulmonary conditions considered for differential diagnosis are acute pneumonias, lymphoblastoma, sarcoid, and pneumoconiosis. We have also seen pulmonary symptoms, productive and slightly bloody sputum, and a positive culture in the face of negative anteroposterior, lateral x-ray films, and a negative bronchoscophic examination.

When systemic disease is suspected, roentgenographic examination of the skeletal system is advisable. The picture of osseous involvement is, like the pulmonary, nonspecific.[73,77,78] The appearance is pre-eminently that of bone destruction with little or no proliferative reaction or new bone formation. The areas are punched out with sharp delineation from the adjacent normal bone. The lesions, however, are less cystlike than those occurring in coccidioidomycosis. In the long bones, these areas are located frequently at or near the epiphyseal lines and the plates may reveal any associated joint involvement. Extensive destruction of the vertebral bodies causes collapse with compression of the spinal cord. A bulging of the psoas shadow suggestive of the presence of a psoas abscess may be present.

Roentgenographically, blastomycosis of the bone may be confused with tuberculosis, sarcoma, coccidioidomycosis, actinomycosis, syphilis, myeloma, and bone cysts.

Clinical Laboratory Examinations.—The findings are those of infection. There is leukocytosis and neutrophilia, particularly in the presence of secondary bacterial infection. The erythrocyte sedimentation rate is increased. When the disease is sufficiently long-standing, hypochromic anemia develops. Blood cultures are very rarely positive, even though Schwarz and Baum[13] in their histopathologic examinations were able repeatedly to demonstrate the organisms in the blood vessels of even uninvolved organs.

PATHOLOGY

Pathologically, North American blastomycosis is a suppurative granuloma with a very close resemblance to tuberculosis. The gross appearance of the skin lesions and the tubercle-like nodules and abscesses in soft tissues and visceral organs is typical of the disease. The pulmonary lesions vary. There may be focal or diffuse pneumonic consolidation, formation of single or multiple abscesses, and miliary distribution of small tubercle-like nodules. Cavities are claimed to be infrequent and usually small, although dimensions up to 7 cm. in diameter may be attained.[75,79,80] Schwarz and Baum[13] encountered a relatively high incidence of adhesive pleuritis, endobronchial lesions, and regional lymphadenopathy. They repeatedly found evidences of scarring and healing.

The bone lesions are essentially destructive in nature, with formation of abscesses, separation of sequestra, and development of chronic discharging sinuses through the overlying tissues and skin.

Dissemination apparently occurs through the lymphatic and hematogenous routes and by direct extension.

Baker[81] has detailed the reaction of the body tissues to invasion by *B. dermatitidis*. This response is essentially granulmatous, with varying combinations of suppuration, necrosis, and fibrosis. The typical microscopic picture is that of a tubercle-like lesion with epithelioid cells, plasma cells, lymphocytes, polymorphonuclear leukocytes, multinucleated giant cells, cellular debris, and the tissue forms of the organism itself. The proportion of the different cellular infiltrates to each other varies from case to case depending more upon the stage of the disease than the location of the lesion. In the more chronic cases, lymphocytic infiltration and fibrosis, showing attempts of healing, predominate; while in the more acute stage, where frank abscesses occur, the polymorphonuclear leukocytes overwhelm the granulomatous infiltrate. The granulomatous nature of the lesion may even be absent, as illustrated by the cases of primary inoculation cutaneous blastomycosis described by Wilson and his colleagues.[52] Histopathologic sections of the skin lesions demonstrate a dense inflammatory infiltrate of lymphocytes and polymorphonuclear leukocytes with numerous blastomycetes. The overlying epidermis in one of the cases was normal, in contrast to the pseudoepitheliomatous hyperplasia commonly observed in the other types of skin lesions.

The mimicry by North American blastomycosis of the tissue reactions of tuberculosis is so close that many authors consider them indistinguishable on a morphologic basis alone. Several,[15,81] however, hold the presence of pyogenic microabscesses and fibrosis as ground for strong suspicion and vigorous search for the casual organism. Caseation necrosis is not so frequent and characteristic as in tuberculosis. The identifying landmark in the histologic picture is the tissue form of the organism which may be seen scattered among the infiltration cells, between the tissue cells, or in the giant cells. The number of organisms in a given lesion varies. The lesion may teem with the organisms or may show rare ones only after diligent examination of many sections. The organisms appear in histopathologic preparations as spherical or oval bodies ranging usually from 5 to 20 μ in diameter. The cell walls are sharply delineated and thick, with a double-contoured appearance. The cytoplasm is granular and vacuolated. Single budding forms are often encountered, although in certain instances they may be very difficult to demonstrate. The daughter bud usually rests on a broad base.

Staining with the periodic acid-Schiff method of Hotchkiss and McManus is a valuable adjunct in the demonstration and study of the organisms in tissue sections.[82,83] It is only supplementary, however, to cultural identification.

The tissue forms of *Blastomyces dermatitidis* must be differentiated from those of the other systemic fungi, *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces brasiliensis*, *Candida albicans*, and *Cryptococcus neoformans*. *Histoplasma capsulatum* is distinguished by its smaller size of 2 to 5 μ in diameter and intracytoplasmic location in the reticuloendothelial elements of the body. On the other hand, the nonbudding, endosporulating spherules of *Coccidioides immitis*, when mature, are much larger with a range of 20 to 80 μ in diameter. *Blastomyces brasiliensis* is also larger, having diameters up to 60 μ and characterized by multiple budding. The buds are connected by narrower processes and may be found all along the periphery of the mother cell. The budding yeast forms of *Candida albicans* are associated with filamentous pseudohyphae. Hyphal elements of *Blastomyces dermatitidis* have not been demonstrated in tissues. *Cryptococcus neoformans* possesses a remarkably thick capsule well seen in India ink preparations.

The distinguishing features cited above are true for typical cases. However, in certain instances differentiation may be very difficult. This is true with histoplasmosis when occasional miniature forms of *Blastomyces dermatitidis*[13,84-88] or giant forms of *Histoplasma capsulatum*[89] do occur. The budding of *Blastomyces dermatitidis* may not be demonstrated, which may cause mistakes in diagnosis inasmuch as the nonbudding forms simulate the immature endospores of *Coccidioides immitis* of the same size. *Blastomyces brasiliensis* with single buds[90] is easily mistaken for *Blastomyces dermatitidis* which, furthermore, may even rarely show multiple budding. *Cryptococcus neoformans* may occasionally be denuded of its thick capsule, depriving the examiner of this differentiating point. These different situations emphasize the essentiality of cultural procedures in recognizing these fungi.

VOL.
5
1957

DIAGNOSIS

As previously stated, there is no diagnostic pattern in the clinical history and manifestations of North American blastomycosis. Even the typical cutaneous granuloma is only suggestive. Clinical diagnosis based on history and physical findings is, at most, presumptive and made unequivocal only by positive demonstration and identification of the causative organism in the laboratory. The procedures are neither elaborate nor expensive, and the only essential requisite is familiarity with *B. dermatitidis*.

Direct Microscopic Examination.—The specimens examined with this procedure are fresh, allegedly infected clinical materials such as pus, sputum, urine, cerebrospinal fluid, scrapings from skin lesions, prostatic fluid and effusions. The clinical means of obtaining these specimens, such as aspiration of subcutaneous abscesses and of joints, spinal tap, prostatic massage, and collection of urine and sputum samples, are standard. Specimens from cutaneous lesions are best obtained by rupturing the small abscesses on the active borders and collecting the exuding purulent material. Weisel and Landis[75] have recommended bronchoscopy as a diagnostic aid in pulmonary blastomycosis. Acree, Decamp, and Ochsner[91] were able to establish the presence of an acute blastomycotic lung abscess by needle aspiration.

Wet mounts of the specimens are prepared on slides with 10 per cent potassium hydroxide in order to "clear" the preparation. Spinal fluid, urine, and other less cellular specimens are mounted directly. These cover slip preparations are examined under the microscope with subdued light. The organisms appear as previously described in stained sections, with the added feature of refractility of the thick, double-contoured walls. They may be difficult to find and may be confused with other cellular elements, air bubbles, or oil droplets. Failure to demonstrate the organism by direct examination of fresh mounts does not necessarily rule out blastomycosis.

Cultural Procedures.—Even when the tissue phase of *B. dermatitidis* is recognized in fresh or stained specimens, it is a wise policy to confirm this by isolation and identification of the organism in cultures. All specimens for laboratory examination should be subjected to cultural procedures. Positive identification of the organism by cultural means from suspected clinical specimens makes the clinical diagnosis practically indisputable in view of the rarity of isolating of *B. dermatitidis* from nature or from uninfected individuals.

The specimens are planted on Sabouraud's dextrose agar for incubation at room temperature and on blood agar or beef infusion dextrose agar at 37° C. The addition of streptomycin and penicillin to the media helps in keeping down bacterial contamination. When contaminants are present, subcultures of suspicious colonies should be made to avoid overgrowth by the faster developing contaminants. The growth generally takes 10 days to 3 weeks to appear, but this period may be as brief as 3 days or may extend to 5 weeks, so that is it advisable to keep the cultures for at least 5 to 6 weeks before discarding.

Blastomyces dermatitidis grows in its mycelial phase at room temperature. It develops as a white, cottony aerial growth or as a folded, membranous colony

with spiny, coremial projections from the surface. The colonies tan with age. Microscopic examination reveals numerous round, oval or pyriform conidia measuring 3 to 5 microns in diameter and borne singly on short lateral sterigmas. These small spores are easily broken off from their attachments and are found lying free in wet mounts made from cultures. At 37° C. on blood agar or beef infusion dextrose agar, the growth is slower. The colonies are small, heaped up, waxy, and relatively easy to pick up from the surface of the agar. Microscopic examination reveals the single budding tissue phase, not infrequently associated, however, with short abortive hyphal filaments. The mycelial phase at room temperature and yeast phase at 37° C. are reversible, depending upon the temperature of incubation. This biphasic character of *Blastomyces dermatitidis* is valuable in its cultural differentiation from other pathogenic fungi, inasmuch as it automatically rules out organisms like *Coccidioides immitis*, *Cryptoccoccus neoformans*, and *Candida albicans*, capable of growing only in one form in ordinary culture media. The other fungi which are biphasic in nature and easily confused with *Blastomyces dermatitidis* are *Histoplasma capsulatum* and *Blastomyces brasiliensis*. *Histoplasma capsulatum* is differentiated by the production of tuberculate chlamydospores in its mycelial phase and the smaller size of the yeast forms. The distinguishing feature of *Blastomyces brasiliensis* is its multiple budding at 37° C. *Sporotrichum schenckii* is also biphasic but the absence of cottony aerial growth and the black pigmentation of its mycelial colonies is characteristic together with the grouped disposition of its conidia. The yeast phase is small and may be cigar-shaped.

Animal Inoculation.—The production of disease in laboratory animals by inoculation of suspected clinical materials or cultures is ordinarily not resorted to for diagnosis. When positive, it becomes a valuable diagnostic tool for it establishes not only the presence of the organism but also its virulence. The mouse has been found most suitable for this purpose. Intraperitoneal or intravenous inoculation is followed by the appearance of suppurative nodular lesions in the liver, spleen, lungs, and lymph nodes. Material from these lesions as well as peritoneal washings show the typical yeastlike tissue forms.

Biopsy.—Not too commonly, suspicion of the presence of blastomycosis is first expressed by the histopathologist upon examination of surgically removed tissues for diagnosis and/or treatment of other conditions, such as tuberculosis or carcinoma. In a number of cases, the diagnosis is made only at the autopsy table. Biopsy is a valuable diagnostic procedure, particularly if the biopsy material is cultured as well as sectioned. Skin biopsies are best taken from the typical borders of the verrucous granulomas. Bronchoscopy, thoracotomy, prostatectomy, and needle aspiration of the liver or lung have been utilized for biopsy purposes.

Skin Test.—Cutaneous hypersensitiveness in a patient with North American blastomycosis is demonstrated by a skin test using a vaccine of the killed yeast-phase organisms or a culture filtrate called Blastomycin. The Blastomycin skin test is akin to tuberculin with a similar technique of performance, reading, and significance. The intradermal injections of 0.1 ml. of standardized antigen in dilutions of 1:10 to 1:100,000, depending upon the degree of sensitivity, re-

sults in the appearance of maximal erythema with central induration in 24 to 48 hours. In very sensitive individuals, if the more concentrated antigen is used, a sterile abscess may develop.[92] In the average patient, it is customary to start with a 1:1,000 dilution of the commercially available Blastomycin.

A positive Blastomycin skin test generally indicates a past or present infection. Although only presumptive diagnostic evidence, the test is particularly helpful in mild or obscure cases or in cases where materials for biopsy or culture are not easily accessible. Its full significance, however, has not been established as yet because of the lack of opportunities to investigate the disease in large series, in contrast to the frequent sizable skin test surveys on tuberculosis, coccidioidomycosis, and histoplasmosis. The results obtained with Blastomycin skin test are not as constant as those of the latter three diseases. The test is negative in the early stages of the disease, in mild localized cutaneous cases, and in the overwhelming or terminal phases when anergy occurs. Disturbing results were reported by Smith[76] in the form of negative results in at least 25 per cent of patients with early proved blastomycosis and positive skin tests in 3 healthy medical students and 10 patients with no evidence of active disease. An important misleading factor is the occurrence of cross reactions with Histoplasmin and Coccidioidin[93] and the antigen of *Blastomyces brasiliensis*,[94] apparently due to a common antigen. Cross reactions between Histoplasmin and Blastomycin are frequent. Patients with histoplasmosis may even give larger Blastomycin skin reactions than patients with proved blastomycosis. Mistakes arising from these cross reactions are obviated, however, if the Coccidioidin, Histoplasmin, and Blastomycin skin tests are performed simultaneously on all patients suspected of these mycotic infections, inasmuch as the skin reactions to the homologous antigens are significantly larger.

Complement Fixation Tests.—The sera of patients with blastomycosis are able to fix complement with suspensions or extracts of *B. dermatitidis* as antigens.[95,96] Like a positive Blastomycin skin test, a positive complement fixation test is only presumptive, but it has been found useful in detecting cases and spurring greater efforts aimed at more definitive diagnosis by demonstration of the organism through mycologic or histopathologic studies. The complement fixing titer is generally zero or low in early, mild, or subclinical infection or in well-localized cutaneous disease. Two of the 4 cases of primary inoculation cutaneous blastomycosis followed by Wilson and associates[52] had no or slightly detectable complement-fixing antibodies. The titer increases with progression of the disease and remains high through the terminal stages.[97,98] In our experience a negative Blastomycin skin test and high complement fixation test signify a poor prognosis. With the recovery of the patient, the antibodies gradually disappear from circulation. The passive transfer of these antibodies from mother to child has been demonstrated by Noojin and Praytor.[66]

Unfortunately, sera from patients with histoplasmosis, coccidioidomycosis, or blastomycosis, both North and South American, cross react.[93,98-102] The major cross-over occurs between histoplasmosis and blastomycosis. It is desirable to perform the complement fixation tests simultaneously with the 3 types of antigens, for the homologous serum generally shows a higher titer.

VOL.
5
1957

Other serologic tests like the precipitin and collodion agglutination tests have not gained general acceptance. The interpretation of their results is more difficult.

DIFFERENTIAL DIAGNOSIS

Because of the protean nature of its manifestations, North American blastomycosis is easily confused with many other conditions. Misdiagnosed cases have reached the autopsy table before the organisms were accidentally discovered. The differential diagnosis depends upon the presenting signs and symptoms of the organ or organs most extensively involved.

The cutaneous lesions are mistaken for tuberculosis verrucosa cutis, lupus vulgaris, nodulo-ulcerative syphilid, gumma, squamous and basal cell carcinoma, mycosis fungoides, iododerma, sporotrichosis, coccidioidal and paracoccidioidal granulomas, chromoblastomycosis, actinomycosis, cryptococosis, trichophytic granuloma, granuloma inguinale, anthrax, tularemia, pyoderma vegetans, yaws, and cutaneous leishmaniasis. The primary inoculation type of cutaneous blastomycosis is to be differentiated from other chancriform diseases such as primary inoculation tuberculosis, sporotrichosis, tularemia, syphilis, yaws, American leishmaniasis, and cat-scratch fever.

The respiratory symptoms associated with the usual onset of systemic (pulmonary) North American blastomycosis simulate the common cold, influenza, or pneumonia. The differential considerations in the more developed systemic disease are tuberculosis, syphilis, primary and metastatic malignancies, pulmonary abscess, pneumonias, sarcoid, lymphoblastoma, pneumoconiosis, multiple myeloma, tularemia, pyemia, osteomyelitis, psoas abscess, histoplasmosis, coccidioidomycosis, South American blastomycosis, sporotrichosis, cryptococcosis, candidiasis, and actinomycosis.

In making the differential diagnosis of blastomycosis, the possibility that it may occur simultaneously in the same patient with other disease such as tuberculosis,[103,104] carcinoma,[105,106] sarcoidosis,[107] and histoplasmosis[87] must be borne in mind.

PROGNOSIS

In untreated cases, the prognosis of the classical cutaneous blastomycosis is good as regards life expectancy, but its chronic recurrent course extends through many years. The risk of conversion into the graver systemic form is ever present, but fortunately rarely occurs. No definite conclusion was drawn by Wilson and his colleagues[52] as regards the prognosis of the cases of primary inoculation type of cutaneous blastomycosis they described, but they were inclined to believe that this form tends to heal spontaneously.

The systemic disease, with involvement of visceral organs, has a foreboding prognosis. Its high fatality rate has been accepted by many on the strength of previous clinical reports. Recently, however, conflicting data have been reported. Kunkel and associates[15] recorded 7 deaths among 25 proved and presumptive cases and the series of 18 cases reported by Sutliff, Kyle, and Hobson[16] included only 2 deaths. The contention that subclinical and/or spontaneously healing North American blastomycosis exists remains to be confirmed in larger numbers of patients.

In the evaluation of prognosis, much help can be obtained from a study of the immunologic picture of the patient. As early as 1939, Martin and Smith[9] suggested the use of the complement fixation titer of the serum as a measure of the extent of infection and the negative skin test as in indication of anergy brought about by extensive, overwhelming disease. In subsequent papers,[97,108,109] they confirmed the value of the complement fixation test and the cutaneous test in prognostication. A favorable outcome is generally expected in patients with a negative complement fixation test and a positive skin test. On the other hand, a high titer of complement-fixing antibodies and a negative skin reaction usually means a grave prognosis.

TREATMENT

The treatment of North American blastomycosis, especially the systemic form, has been unsatisfactory, as evidenced by the multitude of therapeutic modalities used to control the disease. Recently, however, the emergence of a number of promising chemotherapeutic agents is changing the dismal picture.

Cutaneous Blastomycosis.—Skin lesions may undergo complete regression with apparent cure under treatment but the clinical improvement is often only temporary and recurrences are frequent, especially after cessation of therapy.

Since its first use by Gilchrist, iodide therapy has been most extensively used, with some success. Iodides are given in the form of a saturated solution of potassium iodide orally or of sodium iodide intravenously. Andrews[110] found that a mixture of equal parts of ammonium, sodium, and potassium iodides is better tolerated than potassium iodide alone. The importance of determining the cutaneous hypersensitiveness of the patient by skin testing before instituting treatment, especially with iodides, has been repeatedly emphasized in the literature. This is to avoid untoward reactions, and even fatal dissemination, which may occur when a patient with a strongly positive skin test is given idoides. When a positive skin test is obtained, reduction of the cutaneous hypersensitivity of the patient is strongly advised by means of hyposensitization with vaccine before treatment is initiated. Vaccine has also been utilized therapeutically by some authors with variable results. The technical details of iodide therapy and hyposensitization by vaccine are amply described by Conant and co-workers.[111]

Surgical procedures are also of some value in certain types of the disease. Complete excision with or without skin grafting can be done on the smaller, localized lesions. Cauterization, electrocoagulation, cryotherapy or curettage has been combined with iodide therapy and roentgenotherapy, but the greater risk of dissemination accompanying these procedures has been admitted.

Roentgen therapy is another noteworthy therapeutic aid. It has been used to eradicate small, early lesions[112], but is generally combined with iodides and minor surgical procedures. The usual dose given is 75 to 100 roentgen units through 1 to 2 mm. Al once a week for 10 to 14 weeks.[110,113]

The best results are admittedly obtained through combination of iodide therapy, surgical procedures, roentgen therapy, and vaccine hyposensitization. Lesions that are recalcitrant to the above regimes should be treated with the newer chemotherapeutic agents. Local application of silver nitrate, bichloride of mercury, copper sulfate, phenol, and gentian violet are not generally accepted.

Systemic Blastomycosis.—Before the advent of the aromatic diamidines, iodide therapy was the recommended treatment. Its use, however, is empirical and although temporary improvement, and even cure, may occur, it does not essentially alter the course of the disease. It may even affect the disease adversely. Previously, it was widely employed because of the lack of any more efficacious treatment. The reports of successful treatment with the following agents are mainly unconfirmed: undecylenic acid,[80,114] Aureomycin,[115] colloidal copper sulfate,[116] ether,[117] and penicillin.[118]

The most revolutionary development in the treatment of North American blastomycosis was the introduction of the aromatic diamidines. A few members of this group of chemical compounds had been in use for the treatment of trypanosomiasis and leishmaniasis at the time Elson[119] reported the in vitro effectiveness of Propamidine against *B. dermatitidis*. The knowledge gained from the clinical experience with the use of the drugs on these tropical diseases was applied with facility to the treatment of North American blastomycosis. The first attempt to use an aromatic diamidine to control this disease was made in 1950 by Colbert, Strauss, and Green[120] with Propamidine. Stilbamidine was first used with success by Schoenbach and associates[121] the next year although Cherniss and Waisbren[18] recently claimed the fortuituous treatment with stilbamidine of a patient with systemic blastomycosis misdiagnosed as multiple myeloma in 1948. This first report by Schoenbach and his associates was followed by numerous descriptions of clinical experiences on the use of stilbamidine and its closely allied compound, 2-hydroxystilbamidine, in the treatment of North American blastomycosis.[15,16,18,26,40,103,104,122-244] These accumulating experiences testify to the clinical efficacy of these drugs. It is true that remarkable improvement and apparent cures have been attained in a significant number of cases with their use, but they do not control the disease in all cases. Treatment failures, especially in the form of recurrences, have been recorded as more cases are treated and observed for longer periods (Fig. 9).

The pharmacology and mode of administration of stilbamidine and 2-hydroxystilbamidine have been exhaustively reviewed[123,124,131,132,145,146] elsewhere. Further observations of the posttherapeutic course of the cases of blastomycosis treated in the University of Michigan Medical Center with the aromatic diamidines has been described in a previous paper.[142] Table I summarizes the salient features of the poststilbamidine periods in 6 patients. The longest period a patient was observed after the institution of treatment was 4 years and 9 months. The total doses received by Case 2 of 22.5 Gm. of stilbamidine and 21.3 Gm. of 2-hydroxystilbamidine, by Case 5 of 17.8 Gm. of stilbamidine, and by Case 6 of 18.2 Gm. of 2-hydroxystilbamidine certainly are massive in comparison to the average recommended dosages of 3 to 5 Gm. of stilbamidine and 9 to 12 gm. of 2-hydroxystilbamidine. The optimum dosages and mode of administration of these drugs remain to be determined. In the more recent treatment courses given to these patients, the individual doses were increased to 300 mg. stilbamidine and/or 450 mg. of 2-hydroxystilbamidine without any untoward reactions. The relapses in 4 of the 6 patients underscore the occurrence of treatment failures in spite of the relatively huge doses given in futile attempts

to control the disease. The appearance of toxic neuropathy in the same number of patients constitutes another complication of the drug stilbamidine which is claimed to be avoided by the use of the more stable, less toxic but also less effective 2-hydroxystilbamidine. Toxic neuropathy has appeared in 50 to 60 per cent of the reported cases of North American blastomycosis under Stilbamidine therapy and does not seem to be related entirely to the total amount administered. The occurrence of therapeutic relapses and toxic reactions detract from the clinical value of the diamidines in the treatment of blastomycosis. The final answer to the search for the ideal chemotherapeutic agent in the treatment of North American blastomycosis may yet come from the growing list of antifungal antibiotics such as Rimocidin, Fradicin, Mycostatin, and Amphotericin which may parallel the achievements of penicillin, Terramycin, streptomycin, and other antibacterial antibiotics in microbial infections.

OL.
5
957

Fig. 9.—G. G. (Table I) now has had more than 22.5 Gm. of stilbamidine and more than 21.3 Gm. of 2-hydroxystilbamidine (he is now under hospital treatment for a relapsing disease). Although his organism has shown increasing tolerance to the drugs, he still can be controlled by larger doses and prolonged treatment. (From Curtis and Harrell, A. M. A. Arch. Dermat. & Syph. **66**:676, 1952.)

Surgical procedures are of certain value in systemic blastomycosis. Incision and drainage of large pockets of pus is recommended. Urinary obstruction due to prostatic involvement requires surgery. Excision of laryngeal,[147,148] prostatic,[64] and uterine[65] lesions has been attempted. Levitas and Baum[149] regard it worth while to excise isolated, metastatic lesions of the skin or peripheral bones with the possibility of an arrest of the primary focus in the lungs. Even if the visceral lesions remain active, they still suggest extirpation of skin lesions by reason of avoiding cosmetic deformities and eradicating these lesions as foci of further dissemination and of possible contagion.

TABLE I. FOLLOW-UP OF 6 CASES UNDER STILBAMIDINE THERAPY

PATIENT	AGE	SEX	RACE	ORGANS INVOLVED	DRUG GIVEN	TOTAL DOSE (GM.)	FOLLOW-UP PERIOD FROM INSTITUTION OF TREATMENT	NEUROPATHY	THERAPEUTIC OUTCOME
1. A. W.	42	M	C	Lungs and skin	Stilbamidine methionate	3.0	4 yr., 8 mo.	None	No apparent recurrence. Patient admitted in another hospital on June 7, 1956, for diagnosis of advanced pulmonary tuberculosis
2. G. G.	42	M	C	Lungs, skin, bones, joint, prostate	Stilbamidine isethionate 2-hydroxystilbamidine isethionate	22.5 21.3	4 yr., 9 mo.	Present	Recurrent but each recurrence controlled by treatment
3. D. H.	36	F	W	Lungs, skin, subcutaneous tissue	Stilbamidine isethionate	3.3	3 yr., 11 mo.	Present	Recurrent
4. M. D.	37	F	W	Lungs, skin	Stilbamidine isethionate	3.0	9 mo. until June 30, 1953. Patient not heard from since then	Present	No apparent recurrence.
5. E. I.	37	M	W	Lungs, skin	Stilbamidine isethionate	17.8	3 yr., 5 mo.	Present	Recurrent
6. C. R.	47	M	W	Lungs, skin, oral mucous membranes	Stilbamidine isethionate 2-hydroxystilbamidine	2.7 18.2	2 yr., 6 mo.	None	Recurrent

VOL. 5 1957

With greater use of stilbamidine and an acceptance of the concept of the lung being the primary focus, interest in the value of lung resection in the treatment of pulmonary blastomycosis has increased.[15,22,40,80,91,114,135,149-151] Excisional surgery appears reasonably indicated in the few instances where a localized pulmonary focus exists without evidence of dissemination. The usual pulmonary lesions when discovered, however, are often diffuse and do not lend themselves to surgical treatment or may be associated with other visceral foci. Management of pulmonary blastomycosis today is comparable to that of pulmonary tuberculosis where surgical intervention has become a valuable adjunct to chemotherapy. In a like manner, pulmonary resection in blastomycosis is reserved for the residual, irreparably damaged tissues after diamidine therapy. In instances of required diagnostic thoracotomy or therapeutic surgery of misdiagnosed pulmonary lesions, postoperative courses of diamidines are indicated.

Supportive general measures for the blastomycotic patient, such as bed rest and good nutrition, are the same as for any other severe debilitating infection.

OL.
5
957

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VOL.
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VOL 5 195

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VOL.
5
1957

COCCIDIOIDOMYCOSIS

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VOL
5
195

COCCIDIOIDOMYCOSIS (valley fever, desert fever, San Joaquin fever), is an infectious disease produced by inhalation of the fungus *Coccidioides immitis* in a previously uninfected person. The primary phase of the disease process is usually terminated by successful focalization of the initial infection, leaving the person permanently immune to any reinfection. Occasionally, a secondary or disseminated phase of the disease follows the primary infection, with serious consequences to the patient (50 to 60 per cent mortality). The latter and castastrophic complication results from failure of the primary infection to become focalized and takes place most often in susceptible races, i.e., Filipino, Negro, and Mexican. About 5 per cent of patients recovering from clinically apparent primary coccicioidomycosis are left with residual pulmonary changes visible by x-ray, for the most part of cavitary or nodular character. Such residua may require surgical removal.

That the disease is endemic in the Southwestern United States has been well established. That the highly infectious spores of the vegetative phase of *C. immitis* may be carried in the dust-laden valley and desert winds, and thereby gain entrance into the respiratory tract of nonimmune persons, is recognized. The endemicity of the disease itself may depend upon an animal reservoir.[1] Reported observations and studies now under way implicate certain desert rodents which harbor the fungus, *C. immitis*, both in their bodies and in the walls of their underground burrows.[2] That the incidence of infection is related to climatologic conditions in the endemic areas is also evident, with the peak of infection occurring in the dry dusty summer months, especially after the end of a rather wet rainy winter season.[3] Soil cultivation, highway construction, or any similar procedure which disturbs the ground and creates dust, will intensify the infection rate among nonimmune residents and travelers within the endemic areas. These areas have been geographically defined (Fig. 1) and begin with the important southern portion of the great inland California valley called the San Joaquin, extending from there southeasterly to the Mojave desert area and then southward to the Mexican border and probably beyond. To the east the endemic area extends into Southern Nevada, Utah, Arizona, New Mexico, and Southwestern Texas. To the west of the San Joaquin, a few dry valleys in the Coast Range Mountains appear involved. Cases have also been reported from other parts of the Southern

West Coast including San Bernardino, Riverside, San Diego, and Imperial counties. Outside of the United States the dry areas of the Argentine Chaco and Paraguay harbor the fungus.[4] From various other parts of the world isolated instances of infection have been publicized, but are too infrequent to establish any additional foreign endemic zones.

The historical development of our present knowledge of coccidioidal[5] disease begins in this country with the early work of Rixford and Gilchrist in 1896, and Ophuls and Moffitt[6] in 1900, reporting their observations on coccidioidal granuloma (disseminated coccidioidomycosis), and the identification of *C. immitis* as the causative agent. Not until 1937 was there additional important contribution in the work of Gifford and Dickson,[7-9] who described the then unrecog-

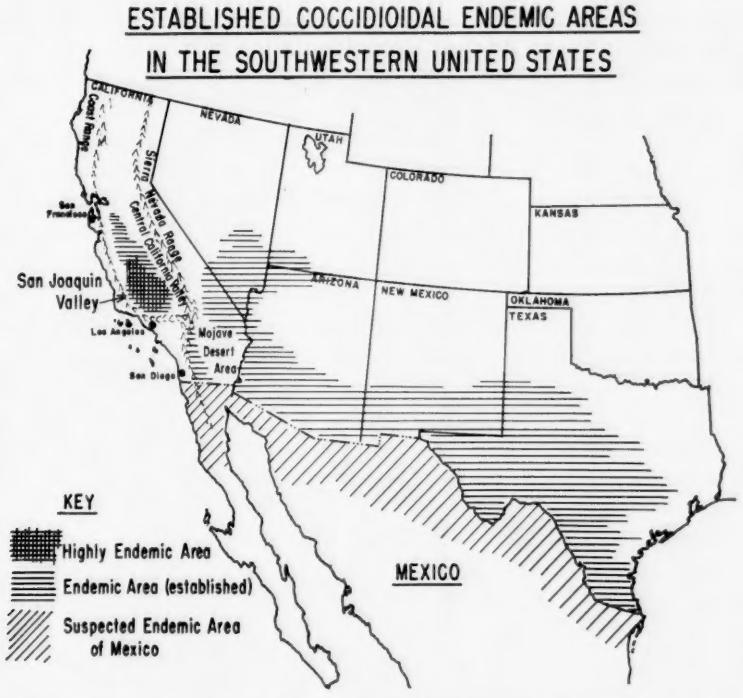

Fig. 1.—The chief areas of endemicity for coccidioidomycosis in the United States.

nized primary phase of the infection, and brought out the relation of so-called valley or desert fever (primary coccidioidomycosis) to coccidioidal granuloma (disseminated coccidioidomycosis). There then followed the important observations of C. E. Smith in the epidemiology of the disease,[10] in the interpretation of the Coccidioidin skin test,[11] and the development of diagnostic serologic tests of great reliability and prognostic significance.[12,13] Descriptions of the variety of primary and residual pulmonary changes seen by x-ray were given by Smith[14] and Winn,[15,16] and medical and surgical treatment was described by the latter.[17]

Coccidioidomycosis is by far the most important of the fungus-produced diseases in the Western United States and remains a continuing problem. The shift

of population to the West Coast, the large flow of vacationists, and the increasing number of military installations in the endemic areas, are some of the factors that make control efforts, and proper recognition and treatment of the disease in its various manifestations, of increasing importance. An exhaustive search for animal reservoirs of infection,[18] a study of racial susceptibility, and continued clinical trial of fungostatic or fungocidal medical agents of promise, remain necessarily urgent. The timely and proper application of acceptable surgical principles of treatment must supplement medical care. Early recognition of the primary infection is of such paramount importance to the patient that every physician must remain constantly alerted to the significance of residence or recent travel within an endemic area, whenever this appears in the history of a patient suffering from an acute respiratory type of infection. Careful medical supervision is still the prime factor in obtaining successful focalization of the initial infection.

Chronic residual pulmonary lesions have been fully described in the literature, with Farness[19] and Winn[20] first reporting cavitation in 1939 and 1941, and Winn and Johnson[15] reporting acute and residual pulmonary changes in 1942. Other authors describing these chronic lesions are Smith,[14] Jamison,[21] and Birsner.[22] A recent review based on 50 patients with benign chronic residual lesions of pulmonary type has been written by Hensler and Cleve,[23] although whether or not all residual lesions can be classified as strictly benign in the ultimate welfare of the patient, should be carefully considered. Certainly, as stressed by these authors, the chronic residual pulmonary changes require careful study to avoid confusion with tuberculosis and neoplastic disease. Also their correct medical or surgical management require, a priori, an understanding by the physician of their origin and potentialities. Repeated pulmonary hemorrhages from residual cavities, the enlarging cavity, the cavity secondarily infected or threatening a transpleural rupture, the abscessing coccidioidoma, all will require careful surgical intervention.

In recent years the liability of an employer or company has been debated in compensation insurance hearings when a susceptible person (such as a nonimmune farm laborer imported for field work within the endemic area) contracts the disease during employment. Also, the insurability of a person with a history of coccidioidomycosis has been questioned, and the medical directors of most insurance companies now require evidence establishing definite arrestment and focalization of primary coccidioidomycosis before insuring a person with a recent medical history of the disease. A history of dissemination will work against insurability because of the high risk of disability or death.

PATHOGENESIS

The causative agent, *Coccidioides immitis*, assumes the vegetative or saprophytic phase of its cycle during growth in the soil of an endemic area. On solid culture medium (such as Sabouraud's agar), it develops in the same way, producing a fluffy, cottony growth formed by delicate branching hyphae, within which are produced the highly infectious chlamydospores. When this mycelial growth is disturbed, the very light spores are carried about by the dust-laden

breezes and winds. Inhalation of this spore-contaminated material produces the primary infection in nonimmune persons. This consists of a granulomatous process, or foreign-body type of tissue reaction provoked by the fungus, as it assumes its parasitic phase of growth within the body environment, which is characterized by the formation of endosporulating spherules. (Fig. 2.) Microscopic study will usually reveal tubercle formation and chronic inflammation that may cause the pathologist to suspect tuberculosis. For histologic clarifi-

VOL.
5
957

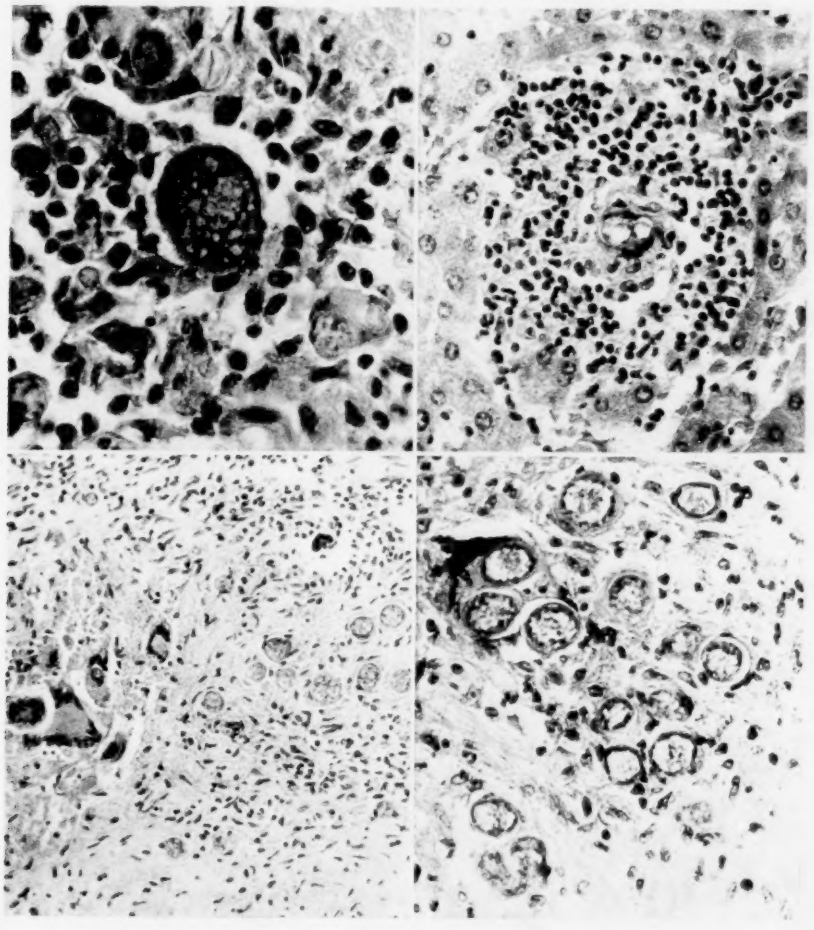

Fig. 2.—*Coccidioides immitis* in human tissues, illustrating the histopathologic response. *A*, *Coccidioides immitis*, parasitic phase. A typical endosporulating spherule present in the human. (×800; reduced ⅓.) *B*, A coccidioidal tubercle. Note giant cell containing spherules of *C. immitis* and surrounding zone of lymphocytes. (×500; reduced ⅓.) *C*, Spherules of *C. immitis* and giant cell formation with chronic inflammatory changes. Heart (subepicardial). (×400; reduced ⅓.) *D*, Coccidioidal meningitis. Note numberous spherules and surrounding lymphocytes and giant cell also containing spherules. (×350; reduced ⅓.)

cation it may be necessary to carry out differential staining of tissue sections, using, for example, carbolfuchsin and the Hotchkiss-McManus techniques, to demonstrate the presence of either the tubercle bacillus or spherules, as the case may be. As the coccidioidal spherule matures, it enlarges to a size easily visible under the high dry magnification of the microscope, and then ruptures liberating the endospores, each of which in turn undergoes spherule growth. This cycle of growth in the host (human or animal), sets up the rapidly reproducing foreign-body type of reaction that, unless successfully walled-off (focalized), will disseminate to other body systems, with a resultant 50 to 60 per cent mortality. Fortunately, dissemination is limited in white-skinned people to less than 0.5 per cent of patients, but in dark-skinned races, (especially the Negro and Filipino), it occurs in 10 per cent or more of the patients.

Primary coccidioidomycosis is often so mild in its onset that in 70 per cent of cases the initial infection is subclinical and unrecognized. The remaining 30 per cent of persons comprise the group who become sick enough to consult their physician. In the subclinical group, only the finding of skin sensitivity to Coccidioidin will reveal that infection has occurred at some previous date. In the clinical group, the severe respiratory illness will usually reveal on chest x-ray areas of pneumonitis, of patchy or nodular infiltrative type. Sometimes hilar lymphadenopathy becomes visible. There may be a primary pleural effusion. These x-ray changes will usually appear in the first week, and occasionally persist for several weeks or months. (Fig. 3.) There may be associated a primary type of cavitation, which closes spontaneously. Pulmonary tuberculosis and lung malignancies may be closely simulated. Twenty-five per cent of the patients in this group develop an erythema nodosum or erythema multiforme type of skin rash. In about 5 per cent of patients these primary pulmonary changes will leave residual manifestations (Fig. 4), including thin-walled cystlike cavities (Fig. 5), and solid "coinlike" pulmonary foci (coccidioidoma).[24] The latter, in turn, will sometimes soften and form localized thick-walled chronic abscesses, another type of residual cavitation. Cavitation may fill or "block off," resulting in solid-appearing foci. Calcified densities, similar to healed primary tuberculous lesions, may appear months later in the lung parenchyma; or involved hilar lymph nodes may undergo calcification. Localized bronchiectatic or fibrotic residua are more unusual.

Such roentgenographic findings in a person who has a history of recent travel through, or residency within, a part of the endemic area, requires the physician to proceed with the Coccidioidin skin test.[11] Sensitivity to this cultural filtrate is determined by the Mantoux procedure, and should be present by the third or fourth week following infection by *C. immitis*. One-tenth cubic centimeter of 1:100 dilution is injected intracutaneously and induration determined at both the 24-hour and 48-hour periods therafter. If erythema nodosum is present, a higher dilution should be used at first, i.e., 1:1,000 dilution. This test is very specific and only when the low dilutions, i.e., 1:10, are used, is there an occasional cross reaction in the already Histoplasmin-sensitive individual. It is necessary that syringes and needles, and pans used for their sterilization, be reserved only for Coccidioidin use, and never contaminated with other skin-testing agents, such

as tuberculin. In patients with disseminated disease, anergy is not uncommon, and in 70 per cent of such instances, there is failure of the skin to react to even a 1:10 dilution. Loss of previous skin sensitivity may also be a bad prognostic sign in the already ill patient.

VOL.
5
1957

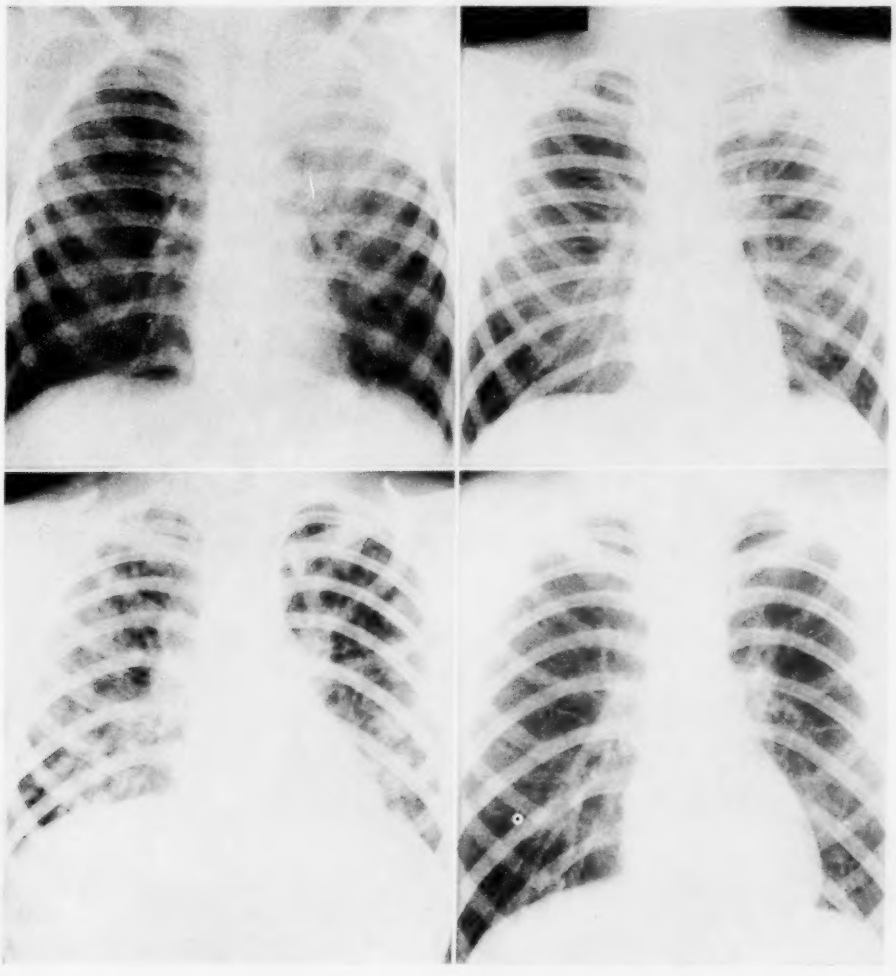

Fig. 3.—Illustrating variations of x-ray findings in primary coccidioidomycosis. *A*, Posteroanterior chest film, June 6, 1952. Primary coccidioidal pneumonitis in a 7-year-old white boy. *B*, Posteroanterior chest film, Sept. 17, 1955. Same patient 3 ¼-years later, illustrating slow but almost complete clearing in the left upper lobe. Serum complement fixation remained high (4 plus in 1:256 dilution) for 2 ½ years, and 4 years later had receded to 3 plus in 1:32 dilution. Patient received Promizole and Daraprim therapy. *C*, Posteroanterior chest film, Oct. 8, 1953. Diffuse nodular infiltrate in 11-year-old white school boy. *D*, Posteroanterior chest film, Oct. 18, 1955. Almost complete clearing after approximately 2 years, a few small calcified densities remaining, right hilum. Precipitin test: 4 plus in 1:40 dilution of antigen. Serum complement fixation: absent at first, appearing in one week; maximum 3 plus in 1:8 dilution being reached in six months, where it has remained. Coccidioidin skin test positive. Received Daraprim and SU-1906 therapy.

SEROLOGY

The serologic tests developed by C. E. Smith and reported in 1950[12] and 1956[13] are most important in the diagnosis and prognosis of coccidioidomycosis.

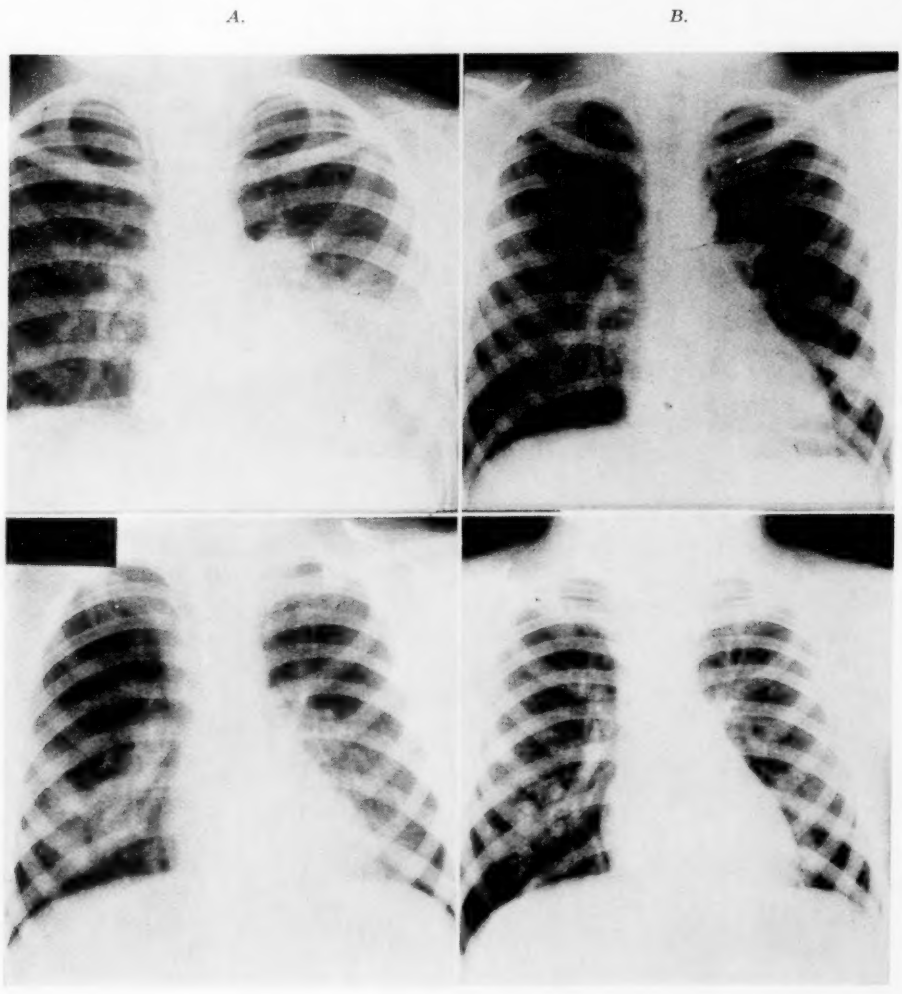

Fig. 4.—Pulmonary lesions due to primary coccidioidal infection and resulting in residual lesions. *A*, Posteroanterior chest film, May 26, 1951. Dense pneumonitis, left lower lobe, in a 5-year-old white boy. *B*, Posteroanterior chest film, Sept. 17, 1955. Four and one-third years later, prominent coccidioidma-type lesion residual in left base, showing early calcific changes. Precipitin test: 3 plus in 1:10 dilution of antigen (May 16, 1951). Complement fixation: 2 plus in 1:2 dilution of serum (May 16, 1951); increasing to 4 plus in 1:16 dilution of serum (Sept. 13, 1951); receding to 4 plus in 1:2 dilution of serum (Nov. 26, 1952); remained sensitive to Coccidioidin and negative to tuberculin. *C*, Posteroanterior chest film, March 7, 1953. Nodular infiltrate, right lower lobe, in a 6-year-old white girl. *D*, Posteroanterior chest film, April 5, 1955, approximately 2 years later, showing residual calcification. Complement fixation: 2 plus in 1:8 dilution of serum; Coccidioidin test positive.

VOL.
5
1957

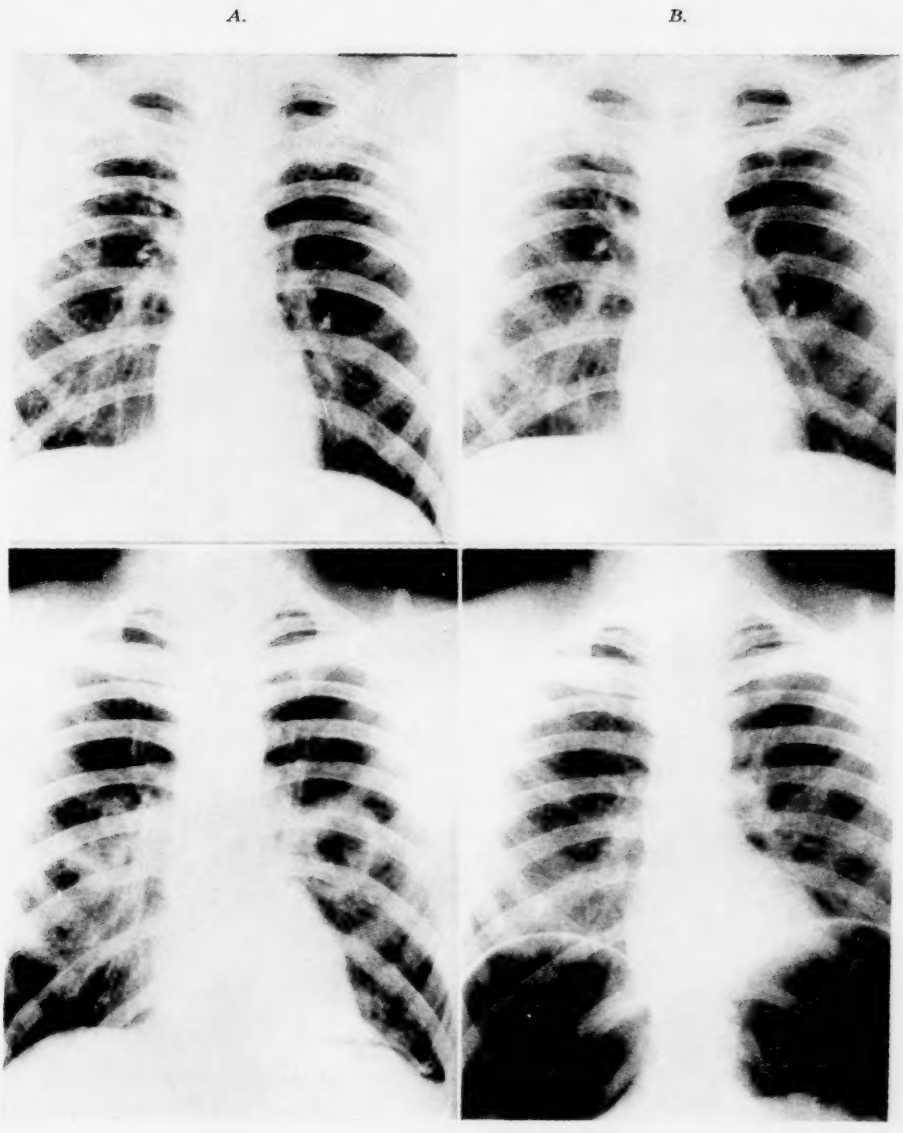

Fig. 5.—Illustrating pulmonary cavitation of residual coccidioidal type, and response to treatment. *A*, Posteroanterior chest film, April 10, 1944. Twenty-five-year-old white male laborer. This x-ray shows typical residual type coccidioidal cavitation on the right. Diagnosis was confirmed by serology and skin tests. *B*, Posteroanterior chest film, March 29, 1949. Five years later, showing complete closure of cavity. This represents spontaneous closure and patient did not receive hospitalization or treatment of any kind. *C*, Posteroanterior chest film, Feb. 25, 1949. Thirty-year-old white male salesman. Large thick-walled residual coccidioidal cavity near left hilum and right perihilar changes. *D*, Posteroanterior chest film, Nov. 25, 1949. Nine months later, pneumoperitoneum present and cavity apparently closed. Pneumoperitoneum had been supplemented by left phrenemphraxis prior to initiation. Skin tests: Coccidioidin (1:1,000) 1 plus; old tuberculin (1:100) negative. Sputum positive for *C. immitis*.

These include complement fixation, using serial dilutions of the patient's serum; and precipitin tests, using a Coccidioidin antigen and undiluted patient's serum. In carrying out the procedure the physician should withdraw 20 c.c. of venous blood, using sterile precautions, later separating the serum aseptically and adding 2 or 3 drops of 1:1,000 aqueous solution of Merthiolate. The experienced technologist, by demonstrating the presence of these humoral antibodies, provides the physician with a conclusive diagnosis. Smith states that these tests are successful in 92 per cent of primary coccidioidal cases who are clinically ill. In the patient with an old pulmonary residual lesion, such as a cavity or coccidioma, the humoral antibodies are so minimal that in only 60 per cent of such patients do serologic tests indicate the diagnosis. Therefore, negative serologic tests do not exclude the possibility of coccidioidal disease, which is directly opposite to the significance of the Coccidioidin skin test. In disseminated disease, serologic studies are practically 100 per cent accurate, whereas, due to anergy, Coccidioidin skin sensitivity may no longer be present.

Only occasionally is it necessary to establish the diagnosis by recovery of the fungus, *C. immitis*, itself. This requires an expert bacteriologist, who fully appreciates the hazards incidental to cultural studies in the laboratory, especially if nonimmune personnel are present. Cultural growth on Sabouraud's medium is easy to obtain from draining sinuses, ulcerated skin lesions, infected spinal fluids, and occasionally sputum. Guinea pigs are quite susceptible, especially if intratesticular inoculation is used. Tracheal wash material and neutralized gastric wash content may be used also for a cultural search for *C. immitis*.

Biopsy of sinus tracts, skin ulcers, and infected lymph nodes may establish the diagnosis. The characteristic tubercle formation and foreign-body giant cell formation do not differentiate between tuberculosis and coccidioidomycosis; tissue stains to demonstrate tubercle bacilli (Ziehl-Neelson), or the spherules of *C. immitis* (Hotchkiss-McManus) are necessary.

SYMPTOMATOLOGY

In the clinical group, the symptoms are those of any respiratory infection, beginning with mild malaise, fever, muscular aching, and cough. Chest pain is frequent and is often substernal in location, and rather persistent. The cough can also be very aggravating and persistent, and is usually only slightly productive. The fever will show afternoon and evening elevation, as high as 103° to 104° F. subsiding considerably in the late night and morning. Malaise may proceed to marked prostration, with considerable weakness and anorexia, and convalescence may be prolonged, with a normal feeling of well-being returning only several months later. Arthralgia may occur, accompanied by some periarticular swelling and stiffness of the joints, especially the knees. Erythema nodosum or erythema multiforme occur in 20 per cent of these clinical cases, and five times as frequently in the female as in the male. The distribution of the nodose lesions and their appearance is much the same as in the rash accompanying rheumatic fever and tuberculosis, consisting of purplish-red elevations of 1 to 6 cm. in diameter, very sensitive or painful to touch, and regressing slowly, leaving purplish-brown areas of temporary pigmentation. They are most frequent on the anterior

surfaces of the lower extremities, extending upward over the outer thighs and hips, sometimes present in the upper extremities, and only occasionally on the trunk. The multiforme type of rash, usually present on upper extremities, trunk and neck, is less frequently seen. Such rashes may be aggravated if simultaneous Coccidioidin skin testing is done. This conforms with the opinion that the appearance of the rash coincides with the development of skin sensitivity and marks on allergic peak. The blood sedimentation rate, almost always elevated, provides a good index by which the course of the infection can be followed and the length of bed rest determined.

TREATMENT

The catastrophe of dissemination requires the attending physician to take every means to prevent its occurrence. The important objective in the initial infection is to apply every therapeutic measure which may assist the patient to achieve focalization of the initial infectious process. Racial susceptibility enters the picture at this point, as in only about 0.2 to 0.5 per cent of white-skinned patients will the disease disseminate. In dark-skinned patients, however, especially the Filipino and Negro, there is a marked susceptibility to dissemination of the infection which occurs 20 to 30 times as often as in the white races. Primary coccidioidomycosis also shows a dangerous tendency to disseminate in the pregnant woman,[25,26] regardless of race, although transmission of the disease to the fetus is practically unknown due to the effectiveness of the placental barrier.

Bed rest of the patient remains the most important therapeutic measure in securing focalization of the primary infection. It should be continued until there is complete regression of fever, a return of the blood sedimentation rate toward normal, clearing or stabilization of the pulmonary x-ray findings, and a lowering or leveling-off of the titer of complement fixation. Again, extra precautions in this respect become necessary when the patient is dark-skinned or pregnant. All of this stresses the importance of the physician keeping in mind the possibility of coccidioidal disease when the medical history reveals recent residence or travel through an endemic area. The roentgenologist plays a most important role when he calls attention to the pulmonary changes, in any of their various forms, as suggestive of coccidioidomycosis.

Careful medical supervision, directed by the above objectives, is all that can be offered at the present time in the prevention of dissemination. In addition to bed rest, there should be included a high protein, high vitamin intake, including 500 mg. of ascorbic acid daily and components of the vitamin B complex, including B_{12} and B_6. Immunotransfusions, or serum globulin preparations from recovered coccidioidal patients with stabilized titers of complement fixation, persisting in serum dilutions of 1:32 or more, may be helpful.

Many drug compounds and antibiotics have been tried and are of no proved effectiveness. Stilbamidine derivatives, given intravenously daily, may be suppressive and are being studied. Amphotercin B may also prove to be effective against *C. immitis*. Promizole, in doses of 12 to 20 Gm. daily, has been thought helpful when given for several months. Chemotherapy should be reserved for threatened or actual dissemination. Recovery from primary coccidioidomycosis confers a solid permanent immunity to further infection.

VOL.
5
1957

When dissemination takes place, all presently known methods of treatment are seriously challenged. Failure to focalize the primary infection results in widespread scattering of the spherules, via lymphatic and vascular channels, to distant body systems (Fig. 6). Dissemination is, therefore, a continuation of the primary phase of infection, of endogenous type, and is not due to exogenous reinfection, or to reinfection from the breakdown of inactive residual lesions, such as pulmonary cavitation or a coccidioidoma. The mortality due to dissemination remains at least 50 per cent and therefore all measures of therapy of any promise should be utilized. This includes aminostilbamidine, 250 mg. given daily intravenously in 5 per cent dextrose solution, for 40 to 60 days. Any abscessing focus must be surgically drained. Ulcerating skin lesions should be repeatedly cauterized with the high-frequency current, usually under general anesthesia. Necrotic bone should be removed, and directly extending abscesses drained. Indurated lymph nodes in the neck should be removed, and suppurating nodes curetted and drained. Occasionally, amputation of a distant focus, such as foot, finger, or leg, is indicated if much destructive disease exists therein. Resection of consolidated lung has been suggested but has not been generally adopted. Collapse measures, including pneumotherapy, are of no proved worth. Drainage of coccidioidal empyema is important, including resection of infected overlying ribs. Chemotherapy of any type, without the proper application of such surgical principles to drain and eradicate areas of suppuration, cannot be effective. Again, supportive treatment including transfusions, adequate nutritional intake, splinting, and careful nursing care becomes important. Coccidioidal meningitis is most serious, and for treatment only adequate cerebrospinal fluid drainage, or replacement with sterile saline, done repeatedly, can be offered. The usual development of block and secondary hydrocephalus often terminates the ordeal. The Torkelson operation has been carried out in one patient to relieve intracerebral pressure by drainage of spinal fluid from the ventricle directly to a detached ureter, obtained by nephrectomy.

Therapy of the group of pulmonary residual lesions again depends on their correct recognition and an understanding of their development and potentialities. About 5 per cent of patients with pulmonary changes associated with primary coccidioidomycosis develop these residual lung changes.

Residual pulmonary cavitation is the most frequently encountered. These thin-walled, cystlike cavities are to be differentiated from the transient cavitation associated with the primary pneumonitis. Residual pulmonary cavities are important because: (1) they may be mistaken for tuberculous cavities; (2) sixty-five per cent of them produce hemoptysis; (3) they can slowly enlarge to form giant cavities which require extensive surgical intervention; (4) two per cent rupture through the visceral pleura, producing secondary pneumothorax, bronchopleural fistula, and coccidioidal empyema; (5) occasionally, they may become secondarily infected, enlarge, and require surgical removal; (6) some, usually those over 2 cm. in diameter, thin-walled and in suitable locations (i.e., not too peripheral because of the danger of rupture), may be closed by pneumothorax or pneumoperitoneum, occasionally supplemented by temporary phrenic nerve paralysis of the hemidiaphragm.

VOL.
5
1957

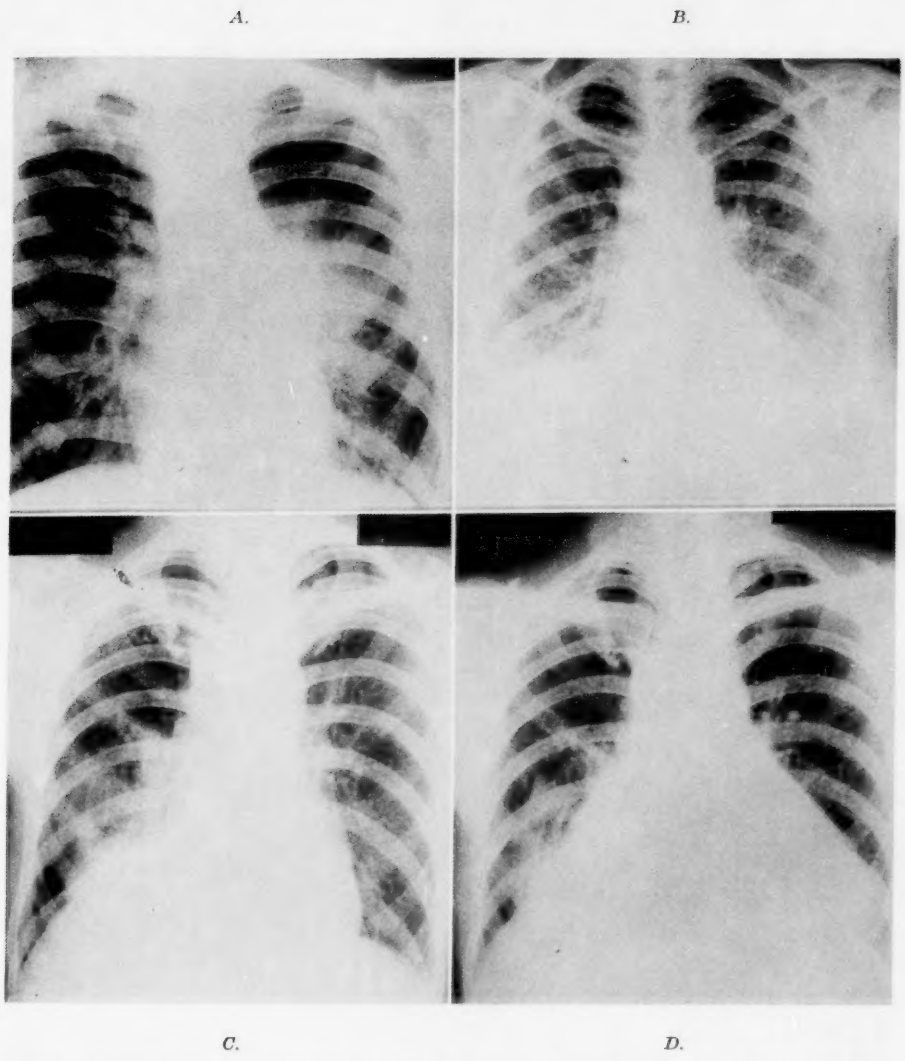

Fig. 6.—Disseminated coccidioidomycosis. *A*, Posteroanterior chest film, April 24, 1940. Thirteen-year-old white male student. Marked left hilar adenopathy with surrounding perihilar pneumonitis. Skin tests: Coccidioidin (1:100) negative, (anergy); old tuberculin negative; sputum positive for *C. immitis*. Death occurred 16 months after onset of illness due to general dissemination of the infection. *B*, Posteroanterior chest film, Nov. 11, 1949. Thirty-four-year-old white housewife. X-ray shows miliary infiltrate throughout both lung fields due to coccidioidal disease. Patient was in fourth month of pregnancy at onset of illness and death followed 5 months later and 2 weeks following delivery. The prognosis of coccidioidal disease becomes serious if it occurs during pregnancy. *C*, Posteroanterior chest film, Jan. 2, 1952. Forty-year-old male Filipino ranch worker. Pneumonitis and consolidation in base of right lung with right hilar adenopathy. *D*, Posteroanterior chest film, Feb. 1, 1952. Same patient. One month later, showing development of marked cardiac dilatation. Death followed 5 months after onset of illness and autopsy revealed coccidioidal myocarditis and pericarditis. Skin tests: Coccidioidin (1:100) 1 plus; old tuberculin (1:100) 2 plus. Serology: complement fixation (1:256) 4 plus; precipitins (1:40) 3 plus.

The coccidioidoma falls into the group of "coin lesions," and is found fairly often as a residual pulmonary change following previous primary infection in the lung. It can also occur as the result of "filling" of a residual cavity, the so-called "blocked" cavity, and persists thereafter as a solid-appearing focus. Occa-

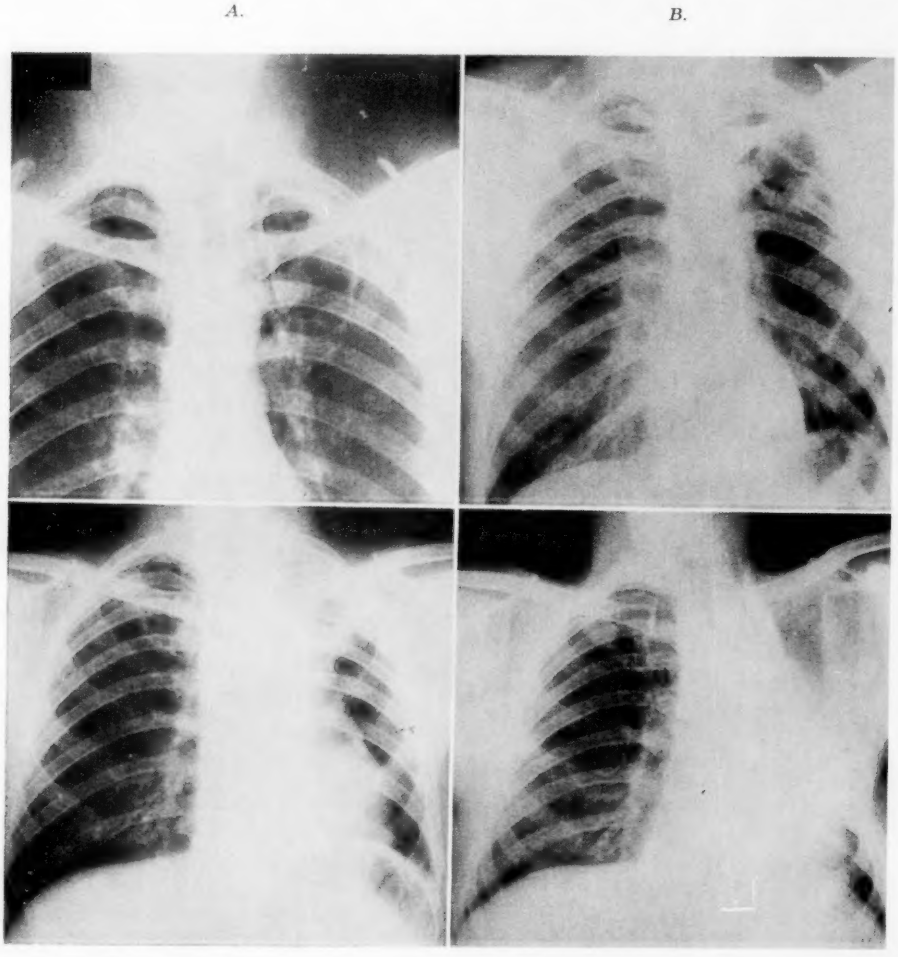

Fig. 7.—Illustrating two of the thoracic surgical approaches utilized in the treatment of pulmonary cavitation of residual coccidioidal type. *A*, Posteroanterior chest film, Jan. 10, 1949. Thirty-six-year-old white male tractor operator. A large residual coccidioidal cavity in the left apex. *B*, Posteroanterior chest film, Nov. 1, 1952. Follow-up x-ray made 3 years and 9 months after surgery (lobectomy). Patient is clinically well. Skin tests: Coccidioidin (1:1,000) 4 plus; old tuberculin (1:10,000) 2 plus. Serology: Complement fixation (1:32) 2 plus; sputum postive for *C. immitis*. *C*, Posteroanterior chest film, July 14, 1950. Forty-year-old white male. X-ray discloses a large cavity in left apex with secondary atelectasis. Note resemblance to tuberculosis. *D*, Posteroanterior chest film, Nov. 15, 1952. Two years and four months later, following extrapleural thoracoplasty. Planigrams disclose no evidence of cavitation. Patient remains well and is working. Serology: Complement fixation (1:16) 1 plus. Skin test: Coccidioidin (1:1,000) 1 plus; old tuberculin (1:10,000) 2 plus. All cultures, gastric and tracheal washes, negative for tubercle bacilli.

sionally, central necrosis takes place, and results in a chronic thick-walled abscess, which then places the lesion in the cavitary group. These thick-walled abscess cavities almost always require surgical removal.

Coccidioidomata can occur as multiple nodular foci, but usually they are single and may closely simulate pulmonary malignancy. They are frequently found in routine chest films of endemic area residents. Their surgical removal and direct study, in case of doubtful diagnosis, is always advisable. In the southern San Joaquin Valley, the coccidioidoma so dilutes the incidence of malignant "coin lesions" that less then 0.5 per cent of the entire group prove to be due to tumor.

The surgical management of residual pulmonary coccidioidal lesions concerns chiefly the cavity and the coccidioma. In general, meticulous care should be used by the thoracic surgeon to prevent soiling or contamination of the pleural space by *C. immitis*. No antibiotic is as yet known to provide the adequate chemical "coverage" that is, for example, available in the surgical approach to tuberculous lesions. Medical obliteration of the small thin-walled cavity is still desirable, if it is not too peripheral in location, by the simple collapse measures previously mentioned. Under conservative management the majority of cavities under 2 cm., and thin-walled, undergo spontaneous closure (Fig. 5). About 50 per cent of residual pulmonary cavities require excision, and such excisional surgery should be wide enough to aviod cutting through surrounding parenchymal lesions (Fig. 7). Lobectomy may therefore be preferred to segmental or local excisions. Prompt obliteration of any remaining pleural space is necessary after pulmonary resection and may require elevation of the diaphragm by pneumoperitoneum. Pneumoperitoneum may safeguard against overstretching of remaining lung tissue, which has been observed and reported[27,28] in relation to the recurrence of coccidioidal cavities following lobectomy and in the remaining lung. This complication has been encountered in three instances by the author.

Melick[29] has summarized the opinions of a group of thoracic surgeons concerning excisional surgery of coccidioidal pulmonary lesions. He found divergence of opinion, with about 50 per cent favoring a conservative approach due to the few but relatively serious complications that sometimes followed excision.

Recognizing that coccidioidomycosis is a continuing and important problem in the Western United States, many observers are reporting progress in recognition and understanding of the disease process. The problem of effective therapy in the uncontrolled disseminated phase of the disease remains a challenge. There is also need for more clearly defined indications for surgical treatment, based on conclusions arrived at only after careful, preoperative study and sufficient follow-up. The low, but still considerable number of postoperative complications reported by many thoracic surgeons suggests continuation of a conservative surgical approach toward pulmonary cavitation in particular.

VOL.
5
1957

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VOL 5 195

VOL.
5
1957

CRYPTOCOCCOSIS

(Torulosis, European Blastomycosis, Busse-Buschke's Disease)

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INTRODUCTION

A YEASTLIKE, budding fungus, usually called *Cryptococcus neoformans*, which forms no hyphal threads but uniquely produces a capsule, exists apparently widespread in nature and occasionally causes cryptococcosis, a chronic, wasting, highly fatal disease in man and animals, characterized by a pronounced predilection for involvement of the central nervous system. It is probable that the incidence of this infection is much higher than is apparent at present, many cases being unrecognized because of lack of sufficient familiarity on the part of the medical profession in general with its mycologic diagnosis and clinical manifestations.

HISTORY

Busse in 1894,[8,9] reported an unusual case of a 31-year-old woman infected with a yeastlike organism; Buschke studied the same patient and added certain details in 1895.[7] Although this case was not typical, there being no involvement of the central nervous system, it is accepted as the earliest record of cryptococcosis. Benham[2] in 1934, after studying the organism (which had been preserved in culture during all those intervening years) concluded that it was identical with other known pathogenic strains of *C. neoformans*. Stoddard and Cutler[50] in 1916 clearly presented the clinical picture and the pathology of this disease, and valuable monographs were contributed by Freeman[26] in 1931, by Cox and Tolhurst[15] in 1946, and by Littman and Zimmerman[39] in 1956.

DISTRIBUTION AND EPIDEMIOLOGY

Cryptococcosis does not appear to be limited geographically, having been discovered in all continents. In this respect it resembles actinomycosis, and differs from coccidioidomycosis, the two blastomycoses, and histoplasmosis. A man may carry the organism about with him while maintaining good health and become infected endogenously only under special circumstances, or he contracts it from some almost ubiquitous source in nature, such as the soil. Both of these mechanisms probably obtain. It would also appear likely that host factors determine whether infection takes place.

Benham[2] found that some strains of Cryptococcus obtained from nature and from normal human skin could not be differentiated from organisms isolated from fatal human infections. Sanfelice[47] discovered pathogenic strains in fermenting fruit juices, as did Klein,[32] Carter and Young[10] in milk, and Emmons from cases of bovine mastitis.[19] Emmons[18] obtained strains pathogenic for laboratory animals from soil, and from the majority of samples of pigeon manure. While no human case has been traced definitely to any of these sources, it seems certain that man contacts the organism frequently. Under similar circumstances actinomycosis is acquired only when that fungus is provided with injured tissue far enough from the body suface to provide anaerobicity, and certain bacteria are also present to furnish an alliance. These factors are not operative in cryptococcosis. At present our only clue lies in the fact that from 10 to 30 per cent of patients with cryptococcosis have lymphoblastomatous disorders, an association which cannot be accounted for statistically by coincidence.

Animals acquire cryptococcosis by natural means, but there has been no proved instance of human infection derived from them. Although transmission from man to man has not been established, Cox and Tolhurst[15] considered it possible after they found that the organisms could be cultured from the sputum in four cases.

The portal of entry of the fungus is only rarely evident, but is probably the lungs in almost all cases. Cox and Tolhurst[15] found that it could resist drying for at least 10 months, and therefore could be inhaled with dust. Respiratory symptoms precede the clinical appearance of cryptococcosis more often than coincidence would explain. Occasionally the gastrointestinal tract has apparently served as the route of inoculation. Percutaneous infection has been assumed by several authors, but the evidence seems inconclusive.

CLINICAL CHARACTERISTICS

Pulmonary Cryptococcosis.—Even though cryptococcosis is associated in the minds of most clinicians with central nervous system diseases, such involvement is often absent,[54] while the lungs, as the probable portal of entry, are always involved at some time during the progress of the disease.[49] There are no characteristics by which pulmonary cryptococcosis can be differentiated from other chronic lung infections, except possibly its tendency to produce fewer symptoms than would be expected from the extent of the lesions revealed by physical signs or by x-ray examinations. Fever is not consistently present, and never severe. Pain or pleural effusion are uncommon. Bronchitis or consolidations, involving the bases rather than the apices and usually bilateral, are indicated by physical signs such as dullness, but there is so little exudate that signs of moisture such as râles are usually absent, and there is but little cough, sputum production, or hemoptysis. Pneumothorax or cavitation are rare. This tendency to remain clinically "silent" in the early stages causes many cases to remain unrecognized unless the better-known central nervous system involvement supervenes. X-rays may suggest neoplasm or lung abscess, but more commonly a diffuse pneumonia.

Some cases of proved pulmonary cryptococcosis have recovered, leading Stoddard and Cutler[50] to suggest that there is considerable tendency toward

recovery, and that the disease may occur frequently in a mild form and heal without recognition, as is known to be the case with some other deep mycoses, notably coccidioidomycosis and histoplasmosis.

Central Nervous System Cryptococcosis.—The invasion of the central nervous system by cryptococcosis is heralded by signs of increasing intracranial pressure or menignitis. Beginning usually in the frontal area, the headache gradually becomes more severe, generalized, and persistent. Occasionally the onset is sudden and violent, with projectile vomiting, clinically suggesting subarachnoid hemorrage. Soon dizziness and neck rigidity appear. Nystagmus, diplopia, and ptosis are sometimes seen. Mental changes are often prominent, the patients becoming irritable, restless, and confused, sometimes simulating a true psychosis.

Cryptococcosis has occasionally been diagnosed from material obtained during operations performed for the removal of "tumors" of the brain or spinal cord. Such focal lesions may occur in any region, and can cause such a variation of symptoms as to cover most of the possibilities involved in neurologic topographic diagnosis. Ataxia and hemiplegia are the most common.

VOL.
5
1957

Fever is not consistently present and is usually mild. The pulse rate is more likely to be depressed by intracranial pressure than elevated by fever. The spinal fluid pressure is increased; the fluid is yellowish and forms a pellicle on standing; the cell count is high and predominantly lymphocytic, and the chloride and sugar levels are decreased. The colloidal gold curve may be normal or of the paretic, tabetic, or meningitic types. Unless the causative fungi are discovered by microscopic study or culture, the clinical picture is entirely consistent with a diagnosis of tuberculous meningitis and has undoubtedly been so accepted on many occasions.

Sometimes there are remissions in the course of the disease, usually short, but it is slowly progressive until death occurs in a few months or rarely after a year or more. There is no recorded instance of recovery once the diagnosis has been established. It is considered possible, however, that many cases of mild infection may occur and recovery ensue without the disease having been recognized.

Cryptococcosis in Other Organs.—There is enlargement of the lymph nodes, spleen, or liver in approximately 20 per cent of cases of cryptococcosis, sometimes closely simulating Hodgkin's disease or another of the lymphoblastomas. This phenomenon was first reported by Fitchett and Weidman,[24] and has since become firmly established as a fact not to be accounted for by coincidence.[29,55] Attempting to explain it, it has been postulated that cryptococcosis may actually be one of the causes of that syndrome called "Hodgkin's disease," or that the capsular substance of the fungus may release substances which can induce "malignant" change. A better theory, however, is that such lymphoblastomatous processes involving the reticuloendothelial system cause it to fail in the performance of some of the valuable duties ascribed to it, such as those involved in the development of specific resistance and immunity, thereby allowing the infection to proceed in an overwhelming manner.

The skin is only occasionally involved in cryptococcosis, the figure being less than 5 per cent of the cases studied.[13,20,28,42,52] Conclusive proof has not accom-

panied claims that the skin was the portal of entry in certain instances. The lesions most frequently reported have been situated on the face and vaguely resemble acne, but consist of papules so translucent as to suggest incomplete vesiculation. Even basal cell carcinoma has been simulated, except that the peripheral border is sloping instead of abrupt. The apices of these lesions become destroyed by erosion, and a sticky, translucent brown or gray fluid is discharged, entirely different from ordinary pus. Cutaneous lesions such as these frequently heal completely.

Collins[14] reported bone involvement in 17 of 200 cases of cryptococcosis, usually multiple osteolytic lesions, especially in bony prominences. Lesions in the bone marrow, large blood vessels, testes, adrenals, kidneys, and occasionally in other areas, have also been reported, and probably would be discovered more often if diligently searched for by pathologists.

Fig. 1.—Cryptococcosis with extensive cutaneous involvement. Note gelatinous material simulating coagulated serum, but in fact consisting of the fungus in almost pure culture. (Courtesy Arthur C. Curtis, M.D.)

PATHOLOGY

The most striking characteristic of cryptococcosis is the tendency for the inflammatory response induced in the host to be surprisingly mild. Frequently, especially in the brain, the body seems not to offer any resistance whatever to the fungal invasion, allowing the organisms to grow almost as if in pure culture. Large masses are thus eventually formed, consisting almost entirely of budding fungus cells, with their large gelatinous capsules, between which only a very coarse reticulum of connective tissue trabeculae and a small number of phagocytic cells are contributed by the host. Around such a mass a thin layer of granulomatous and lymphocytic reaction is seen. Such masses are called "gelatinous tumors"; they exert enough pressure on the surrounding tissues to make room for

themselves, a process formerly thought to be histolytic in nature and due to a lytic "toxin" produced by the fungi, leading Stoddard and Cutler[50] to name the organism (*Torula*) *histolytica*. Single and large or multiple and small, such "cysts" are common in the brain or lungs.

Sometimes there is a more pronounced inflammatory reaction, accompanied by fibrosis and granulomatous changes around small groups of organisms, indicating that some degree of healing is taking place. Caseation is absent in contrast to the tubercles of tuberculosis.

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5
957

Fig. 2.—"Acneform" cutaneous lesion in later stage demonstrating necrotic (but not pustular) center.

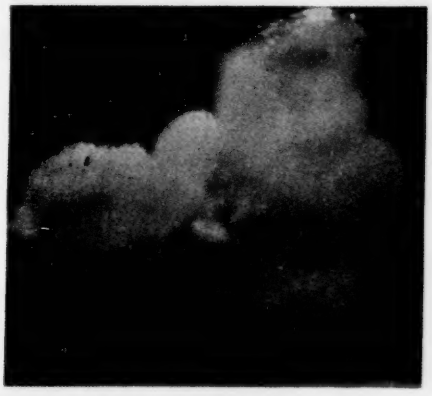

Fig. 3.—*Cryptococcus neoformans* in culture.

Cryptococcus neoformans is usually easily discovered in tissue sections, although it is necessary to realize that the capsular material does not remain after the usual type of fixation and sectioning procedures, being shrunken to a few strands radiating from the cell walls of the fungi. These spaces suggest artefacts, but are too regular in shape to be acceptable as such. In their centers the oval to spherical thick-walled fungus cells are to be seen, staining rather deeply with the basophilic dye. A small proportion will be cut in the appropriate plane to demonstrate the characteristic single thin-walled budlike daughter cell.

MYCOLOGY

A single species of fungus is responsible for cryptococcus, usually called *Cryptococcus neoformans*. Among a large number of other names, *Torula histolytica* and *C. hominis* have been most widely used. This fungus reproduces by producing a single bud at a time from each parent cell, the bud remaining attached until nearly mature in size.

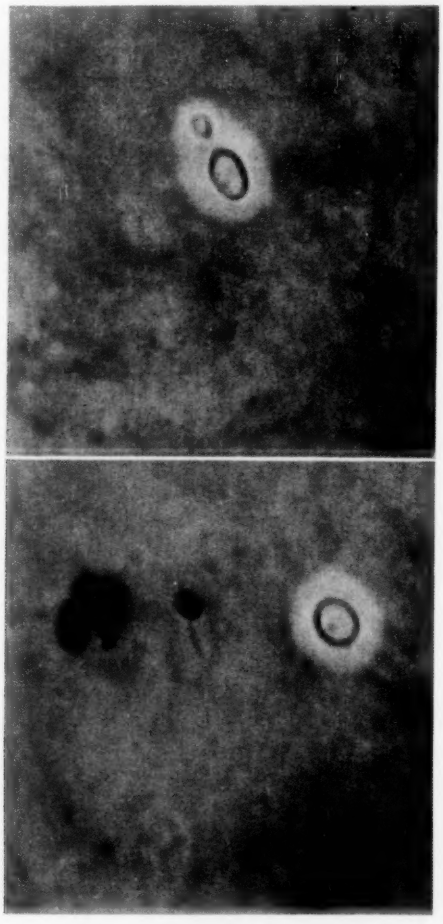

Fig. 4.—*Cryptococcus neoformans* in spinal fluid, prepared by mixing with India ink to render capsule visible. Note the singly budding cell. (Courtesy Marjorie Biddle, Ph.D.)

Direct Microscopic Examination.—Material consisting of spinal fluid or sputum, or that obtained from skin lesions, or aspirated from deeper "gelatinous cysts," may be examined directly to reveal cryptococci. The characteristic capsule is so helpful, however, when revealed by appropriate techniques that they should be routinely employed. The easiest of these utilizes India ink,

VOL 5 195

the granules of which are seen to be pushed aside by the capsular material, allowing it to be easily recognized as a solid transparent mass. Toluidine blue also is serviceable by staining the cell walls. The organisms themselves are simple spherical or ovoid thick-walled cells from 6 to 20 microns in diameter, some of which bear single, thinner-walled daughter cell buds in varying stages of development. Not all cells are encapsulated. Centrifugation of spinal fluid before this examination is helpful.

OL.
5
957

Fig. 5.—*Cryptococcus neoformans* in lung.

Cultural Characteristics.—*Cryptococcus neoformans* is usually easy to cultivate but grows slowly on Sabouraud's glucose agar at room temperature. However, it is advisable to employ also blood agar and incubation of some specimens to avoid failure. Other special media are rarely needed since the inoculum seldom contains much contaminating material such as bacteria or other fungi. Colonies are mucoid at first, soon becoming yeastlike; early the color is white, but later they acquire a yellow, cream, or orange-to-brown tint. Portions of such colonies examined microscopically will reveal the typical thick-walled spherules previously described with a small percentage in the budding process. Most cells will not have capsules; the percentage is higher in older cultures. Some buds will elongate to as much as 30 microns, resembling abortive hyphal threads, thus

revealing the fungus nature of the organisms. In contrast to the "true yeasts" no asci or ascospores are ever produced.

There are numerous nonpathogenic fungi morphologically more or less similar to *Cryptococcus neoformans*, especially Saccharomyces, Rhodotorula, and several species of nonpathogenic Cryptococci. Some strains of the latter are

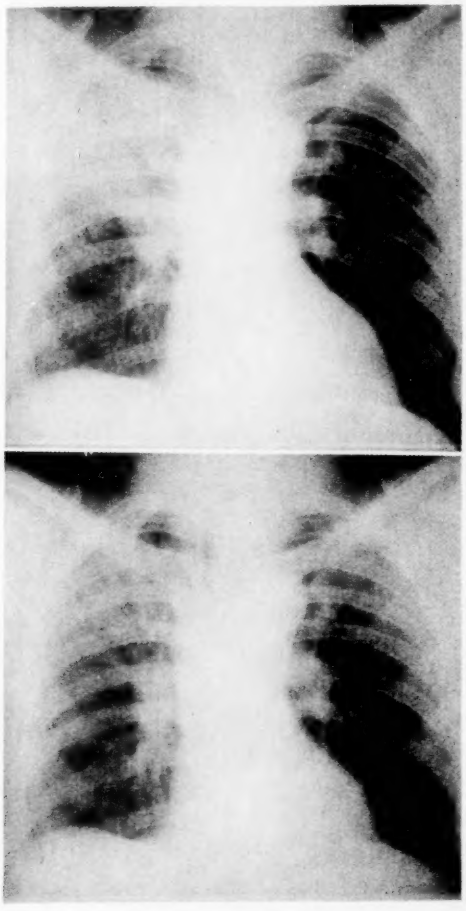

Fig. 6.—*A*, *Cryptococcus neoformans*. In 1951, showing consolidation of right upper lobe with mediastinal lymphadenopathy. *B*, In 1952, partial resolution. Cavities and lymphadenopathy persist. (Roentgenograms courtesy George Jacobson, M.D., and John Wolf, M.D.)

morphologically indistinguishable from the pathogenic *C. neoformans*, and have been termed "*C. neoformans, variety innocuous.*" They usually fail to grow at 37° C. however, which the pathogen will do. Inoculation intraperitoneally into white mice will serve to prove pathogenicity by causing death in from one to four weeks, and revealing at autopsy characteristic gelatinous lesions in the mesentery, lymph nodes, and brain.

VOL.
5
1957

IMMUNOLOGY

Although considerable investigative work has been reported, our knowledge of the immunoallergic phenomena which occur in cryptococcosis is still too fragmentary and confusing for any conclusions to be drawn as to the mechanisms involved. It has usually been stressed that this fungus is but poorly antigenic, perhaps because its large capsule prevents adequate contact of the cell with the tissues and fluids of the host. The large gelatinous cysts with practically no cellular inflammatory reaction surrounding them are striking examples of lack of host resistance; in fact in most cases it is difficult to find evidence that the body is making any effort to resist the disease. This fact, together with the knowledge that *C. neoformans* is encountered frequently by large numbers of persons makes it difficult to account for the rarity of the infection. As with the other deep

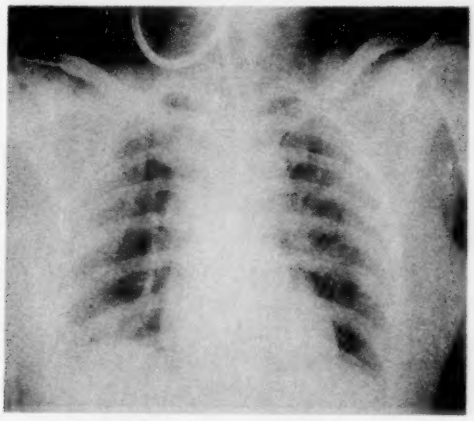

Fig. 7.—*Cryptococcus neoformans.* Solitary toruloma, right lower lobe.

mycoses, the first thought must be that the size of the inoculum may be the determining feature. Certainly mice die routinely from intraperitoneal inoculation, whereas they do not become infected with any frequency by whatever quantities of the same organisms they undoubtedly contact in their natural environment.

It may be that normal persons resist the fungus so completely that no lesion ever results, leading to the conclusion that only those individuals who are abnormal in some important phase of immunologic resistance can acquire the disease. This theory is a favorite one in coccidioidomycosis, despite the lack of such a significant fact to explain the process as we have in the statistical association of cryptococcosis with reticuloendothelial disease.

It is also possible that pulmonary cryptococcosis occurs in large numbers of persons in a form too mild to be recognized as such. Our knowledge of this same stage in coccidioidomycosis and histoplasmosis has been largely derived from studies utilizing their respective skin tests in discovering positive reactors

A. B. C.

Fig. 8.—*A. Cryptococcus neoformans.* In 1948, showing infiltration in right second anterior interspace. *B,* In 1952, slight progression of infiltration. *C,* In 1954, further progression with involvement of entire anterior segment and development, mediastinal lymphadenopathy.

in large masses of "normal" persons. In cryptococcosis such skin tests have not yet been extensively carried out, apparently because the failure to obtain positive reactions in patients known to have the disease has led to the conclusion that the testing material was not "potent." It is possible that this conclusion is entirely in error, since such patients may have developed specific anergy, even as they do when seriously involved with other mycotic disorders.

There have been only a few reports of positive reactions to skin testing with

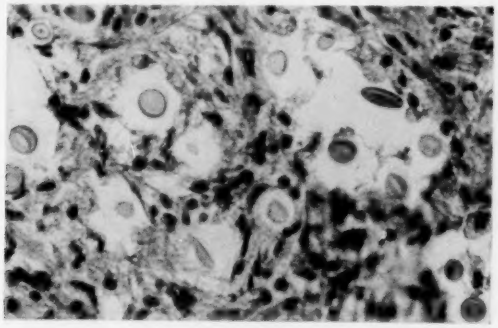

Fig. 9.—*Cryptococcus noeformans* in tissue section. The capsular substance is shrunken during fixation, but its former presence is revealed by the space which surrounds each cell. Note the paucity of cellular infiltrate from the host. (Hematoxylin and eosin stain; low power.)

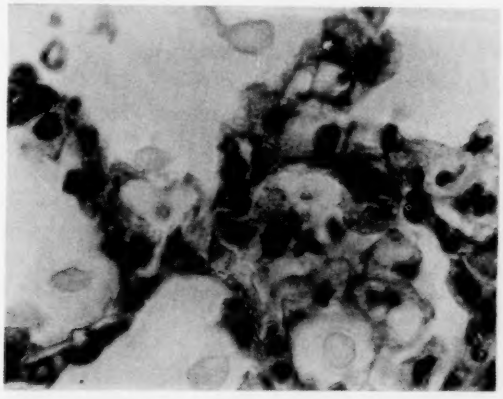

Fig. 10.—*Cryptococcus neoformans* in tissue section. Note the budding cell. (Hematoxylin and eosin stain; high power.)

cryptococcus antigens,[3,17,31,38] and this significance is not as yet apparent. Better purification of antigens (or, perhaps, *less* purification to avoid denaturalization), may yield more valuable testing agents.[21,22,25,33]

Several authors have reported inability to obtain any fixation of complement by using serum from patients with cryptococcosis and antigens derived from *C. neoformans.* It has been suggested that this is because the capsule prevents the organism from engendering the production of antibodies by the host.

VOL.
5
1957

It may be at least equally plausible however, that the antigen is at fault, having been too much changed in some way by the methods used in its extraction.

Agglutination reactions in animals were studied by Cox and Tolhurst,[15] Benham,[2] and by Rappaport and Kaplan.[46] Reactivity in significant dilutions was revealed, but with too much irregularity to be interpreted. Cox and Tolhurst[15] also reported success with a precipitin reaction, but drew no conclusions as to its significance.

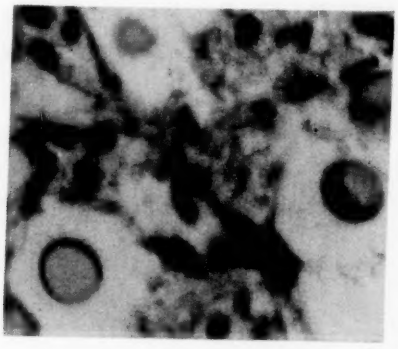

Fig. 11.—*Cryptococcus neoformans* in tissue section.

DIFFERENTIAL DIAGNOSIS

The pulmonary form of cryptococcosis must be differentiated from tuberculosis, sarcoid, malignancy, and lung abscess, as well as other deep mycoses such as actinomycosis, histoplasmosis, moniliasis, coccidioidomycosis, and blastomycosis. The central nervous system type most closely simulates tuberculous meningitis, but brain tumor or abscess, encephthalitis, and involvement with other deep fungous infections must be considered. Cutaneous lesions are easy to identify by microscopic examination provided cryptococcus is suspected.

PROGNOSIS

Pulmonary cryptococcosis may exist in a mild spontaneously healing form, but has usually been of serious importance when diagnosed, proceeding to death in most instances either with or without central nervous system imvolvement. Central nervous system cryptococcosis has been almost uniformly fatal, but may exhibit remissions and recrudescences over from one to several years before conclusion. Cutaneous lesions frequently heal slowly.

THERAPY

There have been reports of isolated cases of cryptococcosis which responded to one or another drug, but failures with the same medicaments when used by others seem to indicate that spontaneous remissions were observed rather than cures. There is, in fact, no treatment which can be relied upon to do more

than palliate. Until recently, large doses of potassium iodide and sulfadiazine have been more favored than other drugs.[40]

Stilbamidine, 2-hydroxystilbamidine, and Propamidine have recently been assayed against cryptococcosis in laboratory animals without benefit by Miller and associates,[41] and by Fisher[23]; but Whitehill and Rawson[51] reported success in one case. A complete summary of the status of chemotherapy and antibiotics in this disease was given recently by Littman and Zimmerman.[39] Nystatin has lately caused much comment, because of some help in mouse cryptococcosis, but failed in two human cases.[39] A few patients have improved after treatment with Actidione but a larger number have failed to improve.[53]

Pulmonary cryptococcosis has been cured on several occasions by being discovered in a sufficiently early stage to permit its complete eradication by surgical lobectomy or pneumonectomy, indicating the need for greater awareness of the possibility of the presence of this disease in pulmonary disorders, and better methods for early diagnosis.[4,27,49] Surgical excision has even been successful on occasion in dealing with localized lesions elsewhere than in the lungs.[1,4,5,6,11,16,28,30,31,36,45]

VOL.
5
1957

The induction of hyperpyrexia has been recommended, based on the observation that *C. neoformans* in culture cannot survive temperatures of 105° to 107° F. for more than 7 days, and some clinical success has been reported.[12,34,35,37] Others have used this method without benefit.[14,39,44] Mosberg and Alvarez-de Choudens[43] combined this with alkalinization, because this fungus does not grow well at high pH, but achieved no success. X-ray therapy has given little cause for encouragement. Vaccinotherapy has been almost uniformly unsuccessful.

Until the subject is clarified, general supportive measures calculated to enhance the body's fighting power must not be forgotten. A high caloric, high protein diet, with supplemental vitamins (especially of the B series), bed rest, and the avoidance of steroid hormones and the usual antibacterial antibiotics are recommended.[48]

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VOL 5 195

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OL.
5
957

SPECIES OF CANDIDA MOST FREQUENTLY ISOLATED FROM MAN: METHODS AND CRITERIA FOR THEIR IDENTIFICATION

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THE increased incidence of all forms of clinical moniliasis, and the implication of monilial species other than *Candida albicans* as agents of disease, suggest the need for a review of the criteria and methods employed in the identification of these species.

C. albicans, the universally accepted pathogen in this genus, can, like the other Candidas, also exist saprophytically on the skin and in the gastrointestinal tract. When it assumes a pathogenic role it may range in virulence from benign to fatal; it may produce lesions that remain localized on the skin or mucous membranes, or become disseminated to invade internal organs. Its pathogenic role was first recognized as the cause of thrush, a disease of the oral mucosa characterized by white soft plaques occurring predominantly in infants and debilitated adults. Although not the basic etiologic factor in sprue, its presence in a rather large percentage of cases is significant, and probably contributes to some of the symptoms of this nutritional disease. A number of common skin conditions have been shown to be due to this species, such as intertrigo, onychia and paronychia, generalized dermatitis, perlèche, superficial glossitis, vaginitis, and vulvar and perianal pruritus. Bronchopulmonary moniliasis may occur as a primary disease, but great care must be excercised in diagnosis, since *C. albicans* may exist in the bronchi as a secondary invader. In conditions such as cancer of the lungs, tuberculosis, diabetes, or even debilitation of the young or old, it is not uncommon to find this microorganism in the sputum; it may then produce definite lesions and contribute to the symptoms, but its mere presence in the sputum does not indicate a mycosis. During recent years a marked increase in the incidence of various forms of moniliasis, particularly those caused by *C. albicans*, has occurred in patients treated with broad spectrum antibiotics, steroids, or by irradiation. Generalized moniliasis in infants, with toxic symptoms and often fatal, also seems to be on the increase. A type of alymphocytosis, fatal to infants, has been described in at least five patients,[1,10,11,15] all suffering from systemic moniliasis. Endocarditis and meningitis terminating in death may also be caused by *C. albicans*.

Other Candidas, particularly *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*, are now isolated with increased frequency from the lesions enumerated above.

†Deceased, Jan. 17, 1957.

The yeasts that are now included in the genus *Candida*, formerly called medical Monilas, are believed to be derived from the genus *Endomycopsis* of the Lower Ascomycetes, by a gradual loss of the ability to form ascospores. The two genera form mycelium or pseudomycelium, as well as blastospores, and ferment sugars strongly, weekly, or not at all.

The taxonomic relationships of the genus *Candida* are thoroughly treated in Lodder and Kreger-van Rij's "The Yeasts"[20] in which the yeasts that do not form ascospores are placed in the family Cryptococcaceae of the class Fungi Imperfecti. These authors subdivide this family into three subfamilies: (1) Cryptococcoideae (budding cells, may or may not form mycelium, no arthrospores, no carotenoid pigments); (2) Rhodotoruloideae (carotenoid pigments, no mycelium, budding cells); (3) Trichosporoideae (no carotenoid pigments, mycelium, budding cells, arthrospores).

The subfamilies Rhodotoruloideae and Trichosporoideae have one genus each, whereas the subfamily Cryptococcoideae is further subdivided on the basis of the formation or lack of formation of mycelium, etc., into seven genera, one of which is the genus *Candida*.

Diddens and Lodder[9] designated *Candida albicans* as the type species. The synonymy and history of the genus and its species are discussed fully by Lodder and Kreger-van Rij[20] who list 87 synonyms for *C. albicans* alone. The classification of the genus according to these authors is as follows: "Phylum Thallophyta; Class Fungi Imperfecti; Family Crytococcaceae; Subfamily Cryptococcoideae; Genus *Candida*."

As with the Cryptococci, no one criterion is sufficient for the identification of the Candidas. We have found in the past and still maintain that morphology, biology, serology, as well as pathogenicity for laboratory animals must be considered. Different groups of workers use slightly different methods for the study of these forms, but despite these differences in technique, most agree as to the essential facts. Although Lodder and Kreger-van Rij[20] list 30 species of *Candida*, there are 8 clear-cut species which are isolated from man with relative frequency, and it is with the identification of these 8 species that we shall deal in this paper. They are as follows:

1. *C. albicans* (Robin, 1853) Berkhout, 1923.
2. *C. tropicalis* (Castellani, 1910) Berkhout, 1923.
3. *C. stellatoidea* (Martin, 1937) Langeron and Guerra, 1939.
4. *C. parapsilosis* (Ashford, 1928), Langeron and Talice, 1932.
5. *C. krusei* (Castellani, 1910) Berkhout, 1923.
6. *C. guilliermondii* (Castellani, 1912) Langeron and Guerra, 1938.
7. *C. pseudotropicalis* (Castellani, 1910) Basgal, 1931.
8. *C. lipolytica* (Harrison, 1928) Diddens and Lodder, 1942.

METHODS

Direct Examination of Tissues.—For the direct examination of tissues or exudates for the presence of Candidas, scrapings or a drop of the exudate should be mounted in 10 to 30 per cent NaOH on a slide, a cover slip placed on this, and the preparation gently heated. Microscopic examination will reveal oval or

round budding cells which are frequently clustered along mycelial elements near the hyphal septa (Plate I, Figs. 1 and 2). The Gram stain is useful in examining sputum or exudates, revealing the organisms as gram-positive. The periodic acid-Schiff stain[19] is sometimes useful in examining scrapings from the skin or mucosal lesions, or tissue sections, the organisms staining bright red or magenta (Plate I, Fig. 3).

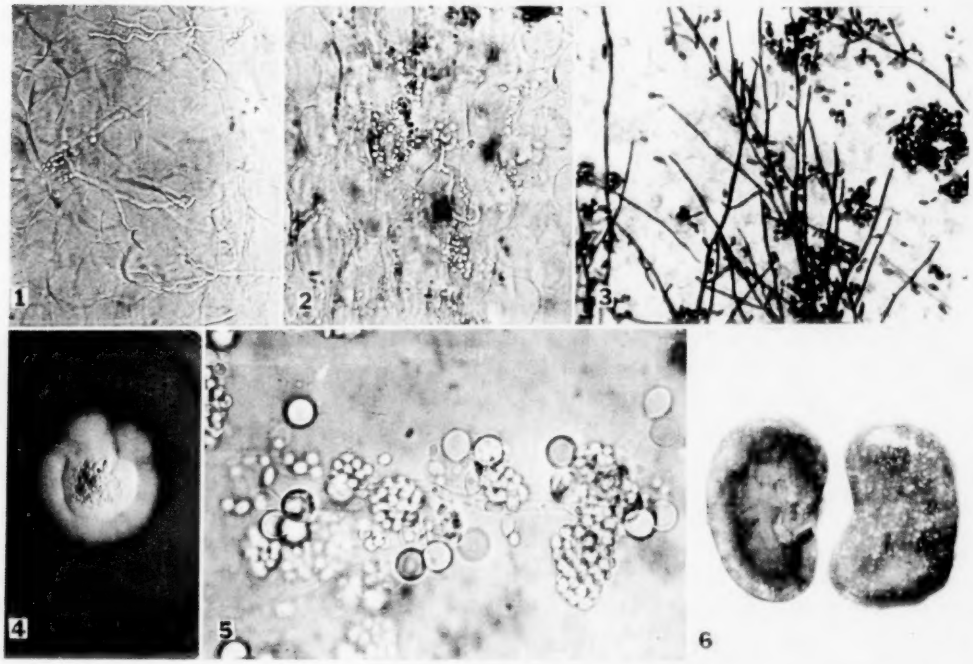

Plate I.—*Candida albicans*. Figs. 1 and 2, Budding cells and mycelial elements in epidermal scrapings, NaOH preparations. (×430; reduced ⅜.) Fig. 3, Budding cells and branching mycelium in urine sediment, stained by Periodic acid-Schiff method. (×430; reduced ⅜.) Fig. 4, Giant Colony on Sabouraud's honey agar, after one month at room temperature. (×1; reduced ⅜.) Fig. 5, Unstained preparation from corn-meal agar culture showing clusters of blastospores (budding cells) along the hyphae, and terminal chlamydospores (single, larger, thick-walled spherical cells), sometimes preceded by "sterile cells." (×1,000; reduced ⅜.) Fig. 6, Miliary abscesses in kidneys of experimentally infected rabbit. (×1; reduced ⅜.)

Isolation.—Cultures should be made by planting bits of tissue or streaking exudates on slants or plates of Sabouraud's glucose agar to which antibiotics may be added if bacterial or mold contamination is suspected. The yeast colonies, which are usually soft and creamy, appear in 2 or 3 days to a week, at which time single colonies should be fished and transferred to fresh slants, or purified by plating, using any of the serial dilution techniques. Individual, well-separated colonies can then be subcultured in pure form for further study.

Morphologic Studies.—The morphology of giant colonies, although not diagnostic, is sometimes helpful in identification. It is obtained by inoculating an

agar plate in the center at a single point with the purified strain and incubating at room temperature for a month. Sabouraud's glucose or honey agar, or malt extract agar are used for this purpose. It must be mentioned here that colony morphology of the Candidas shows great variation from strain to strain within a single species, and that this variation even with constant conditions of growth may be so great as to overlap species boundaries. It is for this reason that it is repeatedly emphasized that one criterion alone, and this one in particular, is not sufficient for specific differentiation. The only two species having more or less characteristic colony texture and pattern are *C. krusei* and *C. lipolytica*, as described elsewhere in this paper. For microscopic morphology, corn-meal agar plates are used, inoculated by cutting a streak into the agar across the surface of the plate. Growth on this medium, which is usually ready for examination in 2 days to a week, serves to separate the Candidas from other yeasts or Cryptococci. The Candidas develop mycelium which extends as a fringe, visible to the naked eye, on either side of the line of inoculation. This at a glance permits elimination of the nonmycelial yeasts which grow along the streak in a smooth line. For more detailed examination of the microscopic morphology, the corn-meal plates should be examined by inverting them on the stage of the microscope under the low-power objective, or by cutting out small portions of the growth with a needle and mounting on a slide for high-power examination.

VOL.
5
1957

It should be mentioned here that the choice of corn-meal agar as well as the temperature of incubation is important for the production of characteristic morphology. The presence of too much sugar in the medium, as well as incubation at 37° C., favors the production of blastospores and inhibits the production of filaments and chlamydospores essential for the identification of certain species. A number of alternate media have been recommended[6,7,14,16,23,26,29] and some are commercially available for the study of microscopic morphology of the Candidas. It has been found, however, that a homemade medium, prepared from unrefined corn meal[4] with incubation at room temperature, is far superior for the rapid expression of the diagnostic morphologic pattern than other methods or media suggested. (Benham and others, unpublished observations.)

Biochemical Reactions.—The ability to ferment or assimilate certain sugars are useful criteria for distinguishing between species. To study the fermentation reactions, the carbohydrate broth with bromthymol blue indicator recommended by Martin and co-workers[22] is employed in this laboratory, but instead of overlaying the broth with petroleum jelly for trapping the gas, the Durham type of fermentation tube is used. Incubation is at room temperature for 14 days; at the end of this time a test is considered negative if no acid or gas has been produced. Four sugars are sufficient for these tests: glucose, maltose, sucrose, and lactose. For the sugar assimilations, determined by auxanograms, the technique of Beijerinck[3] and that of Benham,[5] are followed, 6 sugars being adequate for differentiation: glucose, lactose, sucrose, galactose, raffinose, and maltose.

Agglutination Reactions.—The agglutination and agglutination-absorption tests, as shown by Benham in 1931,[4] are additional useful criteria for separating or grouping certain *Candida* species. Immune sera are prepared and tests are performed by essentially the same techniques described in 1931, with slight

Plate II.—Giant colonies of various isolates of each of the 8 species of *Candida*, after one month at room temperature on malt extract agar. (×1; reduced ⅔.) Figs. 1-4, *C. albicans*. Fig. 5, *C. tropicalis*. Figs. 6 and 7, *C. stellatoidea*. Figs. 8-10, *C. parapsilosis*. Figs. 11 and 12, *C. krusei*. Fig. 13, *C. guilliermondii*. Fig. 14, *C pseudotropicalis*. Fig. 15, *C. lipolytica*.

modifications such as the use of 0.5 per cent formalin for killing the cells to be injected into animals; 0.01 per cent Tween 80 to obtain more homogeneous yeast-cell suspensions for agglutination or absorption tests; and 0.005 per cent Merthiolate for preservation of the immune sera for storage in the refrigerator. Serial twofold dilutions of the sera, ranging from ¼ through 1/2,048, are employed.

Animal Pathogenicity.—For the determination of pathogenicity of Candida strains, the rabbit and the albino mouse are used. The intravenous route of injection is employed, the usual dose being 0.1 ml. of a 1 per cent suspension by volume of packed cells of the yeast. Cells are obtained by washing off with sterile saline the growth from 24- to 48-hour Petri plate cultures on Sabouraud's glucose agar and centrifugalizing for 15 minutes at 1,500 r.p.m.

VOL.
5
1957

CHARACTERISTICS OF THE EIGHT SPECIES

1. *Candida albicans* (Robin, 1853), Berkhout, 1923.—

a. *Morphology:* The giant colonies of *Candida albicans* are characterized by a cream-to-white, pasty, heaped-up growth, smooth or corrugated at the center, or covered by ruptured air bubbles. A mycelial fringe in the depth of the agar along the edge of the colony is occasionally seen. After one month on Sabouraud's glucose or honey agar or on malt extract agar, colony diameter is about 40 mm. (Plate II, Figs. 1 to 4.)

The microscopic morphology of *C. albicans* on corn-meal agar (Plate I, Fig. 5; Plate III, Fig. 1) shows true mycelium as well as pseudomycelium with characteristic spherical clusters of blastospores, i. e., budding oval to round cells, occurring at the level of the hyphal septa and giving the suggestion of beads on a string. Chlamydospores, i. e., terminal, sperical, enlarged cells with a thick wall and one or more coarse refringent droplets in the cytoplasm, are characteristic of *C. albicans*, and sometimes considered diagnostic for the species. They occur singly or in clusters and may be preceded on the hypha by one or more spherical cells with thin walls and a hyaline cytoplasm. These subterminal cells are referred to as sterile. Diagnosis of this species on the basis of chlamydospores alone should be avoided since *C. tropicalis* and *C. stellatoidea* also produce similar structures.

b. *Biochemical reactions: C. albicans* produces acid and gas on glucose and maltose, occasionally acid but no gas on sucrose, and no reactions on lactose. The species assimilates glucose, maltose, sucrose, and galactose.

c. *Agglutination reactions:* When antisera are prepared by the methods described, agglutinin titers, ranging from 1:640 to 1:2,048, are obtained with the homologous strain. There is strong cross agglutination with other strains of *C. albicans*, strong to moderate with strains of *C. tropicalis* and of *C. stellatoidea;* weak to negative with the other 5 species under discussion. By these tests, a number of closely related types of *Candida* are encountered, some strains being difficult to place by this criterion alone. Three species, however, can be separated distinctly on this basis: *C. albicans*, *C. parapsilosis*, and *C. krusei.*

d. *Animal pathogenicity: C. albicans* is lethal to rabbits; upon intravenous inoculation by the method described, death occurs in 4 to 5 days with definite

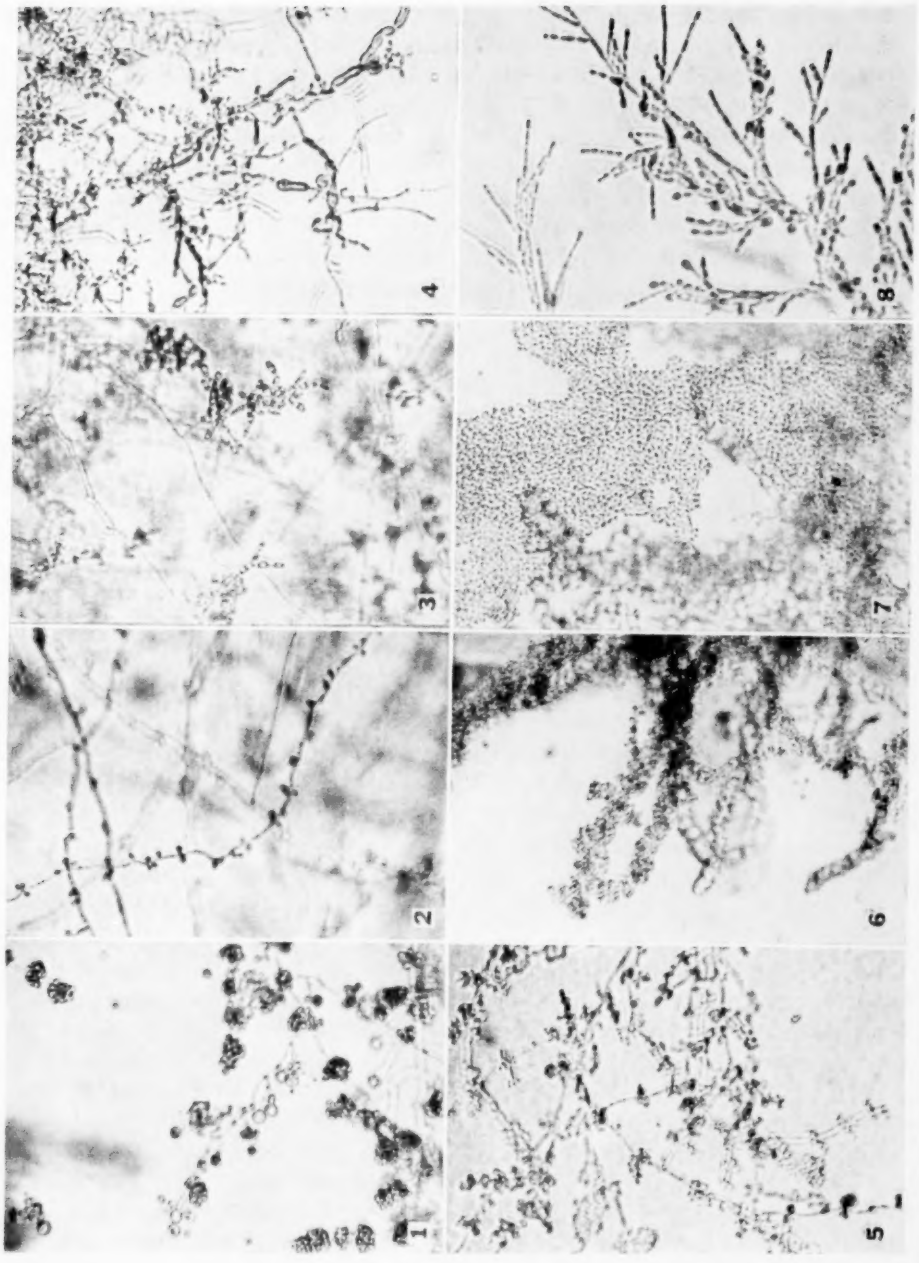

Plate III.—Culture mounts of 8 species of *Candida* on corn-meal agar showing microscopic morphology. (×430; reduced ⅓.) Fig. 1. *C. albicans*. Fig. 2. *C. tropicalis*. Fig. 3. *C. stellatoidea*. Fig. 4. *C. parapsilosis*. Fig. 5. *C. krusei*. Fig. 6. *C. guilliermondii*. Fig. 7. *C. pseudotropicalis*. Fig. 8. *C. lipolytica*.

VOL 5 195

abscesses in various organs, particularly the kidneys. In the latter, miliary abscesses are concentrated in the cortex and are visible as raised white dots surrounded by hemorrhagic areas (Plate I, Fig. 6). Examination of these abscesses reveals the presence of budding cells and mycelial fragments, and *C. albicans* can be recovered in cultures from these lesions.

2. *Candida tropicalis* (Castellani, 1910) Berkhout, 1923.—

a. *Morphology:* The colony is somewhat faster growing than *C. albicans*, reaching a diameter of 45 to 60 mm. at the end of 3 weeks (Plate II, Fig. 5). It is more membranous, being heavily wrinkled, and of a deep cream color. On corn-meal agar, the microscopic morphology shows great development of mycelium with budding cells near septa and between septa, sometimes singly, sometimes in small clusters (Plate III, Fig. 2). There is only an occasional chlamydospore and these are irregular in size and shape.

b. *Biochemical reactions:* On glucose, maltose, and sucrose, acid and gas are produced; the auxanogram is identical with that of *C. albicans* on the six sugars employed.

c. *Agglutination reactions:* High titers are obtained in homologous antiserum; strong cross agglutination with *C. albicans* and *C. stellatoidea;* weak to none with the other five species.

d. *Animal pathogenicity:* This species generally induces only a nonfatal torticollis with healed lesions in the brain and kidney of the rabbit; 50 per cent mortality in the rabbit was reported by Drouhet and Couteau.[13]

3. *Candida stellatoidea* (Martin, 1937) Langeron and Guerra, 1939.—

a. *Morphology:* This species was not described until 1937,[22] probably due to the fact that its morphology is so similar to *C. albicans* that it requires other differential factors to distinguish between these 2 species. The giant colonies are strikingly alike (Plate II, Figs. 1 to 4, 6, and 7). The microscopic morphology is also very close, but there are some tendencies that may aid in their differentiation. In *C. stellatoidea* the clusters of blastospores are usually less regularly spherical and the chlamydospores are less regular in shape and usually formed with more than one sterile cell in them (Plate III, Fig. 3).

b. *Biochemical reactions:* This species ferments the same carbohydrates as *C. albicans*, producing acid and gas on glucose and maltose. In our experience acid is sometimes observed on sucrose also, though this is not in agreement with Conant.[8] In auxanograms the species differs from *C. albicans* in not utilizing sucrose.

c. *Agglutination reactions:* This species shows strong to moderate cross agglutination with *C. albicans* and with *C. tropicalis.*

d. *Animal pathogenicity:* A nonfatal torticollis with healed lesions in the brain and kidneys is produced in rabbits.

4. *Candida parapsilosis* (Ashford, 1928) Langeron and Talice, 1932.—

a. *Morphology:* This species is quite different from the preceding three. The colony is somewhat smaller, raised, wrinkled, and white or cream in color, being, after one month, 22 to 24 mm. in diameter (Plate II, Figs. 8 to 10). Microscopically, its mycelium and blastospores are smaller in diameter than those of *C. albicans*, except that, in addition to this fine growth, certain filaments and

blastospores are much coarser, about twice the size as the ordinary ones (Plate III, Fig. 4). These are referred to as "giant mycelium," and serve as a guide in identifying this species.

b. *Biochemical reactions:* This species produces acid and gas on glucose only; assimilates the same sugars as *C. albicans.*

c. *Agglutination reactions:* This species shows moderate cross agglutination with *C. stellatoidea* and none with the other 6 species.

d. *Animal pathogenicity:* This species has no pathogenicity for the rabbit by the usual methods of inoculation.

5. *Candida krusei* (Castellani, 1910) Berkhout, 1923.—

a. *Morphology:* This species forms a colony which usually distinguishes it at a glance from the other Candidas. It is flat, dull, membranous, and sometimes wrinkled, rather fast growing, reaching a diameter of about 60 mm. in 3 weeks, though the size of the colony may vary (Plate II, Figs. 11 and 12). The microscopic morphology on corn-meal agar is also quite characteristic; the blastospores are long-oval or nearly cylindrical and occur on the branches as "crossed match sticks" (Plate III, Fig. 5).

b. *Biochemical reactions:* On glucose only are acid and gas produced. The auxanogram differs from that of any of the other strains; sucrose, maltose, and galactose are not assimilated.

c. *Agglutination reactions:* This species shows little or no cross agglutination with the other species, indicating, as do its other characteristics, a more distant relationship to *C. albicans* than that shown by *C. tropicalis* and *C. stellatoidea.*

d. *Animal pathogencity: C. krusei* is not pathogenic for rabbits tested as outlined above.

6. *Candida guilliermondii* (Castellani, 1912) Langeron and Guerra, 1938.—

a. *Morphology:* This form is very similar to the *C. albicans* group in some respects. The colony is much the same in size and appearance, measuring usually 30 mm. at one month (Plate II, Fig. 13). The microscopic morphology shows a similar, though smaller pattern than *C. albicans,* with ball-like clusters of blastospores. No chlamydospores are produced (Plate III, Fig. 6).

b. *Biochemical reactions:* On glucose and sucrose only are acid and gas produced. In the auxanogram lactose is the only sugar not assimilated.

c. *Agglutination reactions:* This species shows strong cross agglutination with *C. pseudotropicalis* and *C. lipolytica,* weak with *C. stellatoidea,* none with the other species.

d. *Animal pathogenicity:* This fungus is nonpathogenic for rabbits.

7. *Candida pseudotropicalis* (Castellani, 1910) Basgal, 1931.—

a. *Morphology:* This species has a somewhat similar colony pattern to that of *C. guilliermondii,* its size approximates that of *C. albicans* (Plate II, Fig. 14). Microscopically, the growth consists of elongated cells which readily fall apart (Plate III, Fig. 7).

b. *Biochemical reactions:* This species produced acid and gas on glucose, sucrose, and lactose. In the auxanogram, maltose is the only sugar not assimilated.

c. *Agglutination reactions:* This species does not show cross agglutination with any of the other 7 species.

VOL 5 195

d. *Animal pathogenicity:* This fungus is nonpathogenic for rabbits.

8. *Candida lipolytica* (Harrison, 1928) Diddens and Lodder, 1942.—

a. *Morphology:* This species, like *C. krusei*, is one that can be readily recognized by its colony which is yellowish, glistening, and corrugated, being about 50 mm. in diameter at 4 weeks (Plate II, Fig. 15). Its microscopic morphology is also quite distinct, consisting of pseudomycelium or true mycelium and sparse, elongate blastospores, verticillate or in chains (Plate III, Fig. 8). Because of this and other characteristics, doubt has been expressed by some students of this group as to whether or not *C. lipolytica* belongs in the genus *Candida*.

b. *Biochemical reactions:* No gas is produced on any of the four sugars; it assimilated glucose only.

c. *Agglutination reactions:* There is no cross agglutination with any of the other seven species.

d. *Animal pathogenicity:* There is no pathogenicity for the rabbit.

A summary of the characteristics of the 8 species of Candida that form the subject of this paper is presented in Table I.

It will be noted from this table that *C. albicans*, *C. tropicalis*, and *C. stellatoidea* show many characteristics in common and fall more or less into one group. The 3 show some degree of virulence for the rabbit, *C. albicans*, upon intravenous injection of 0.1 c.c. of a 1 per cent suspension by volume, kills in 4 to 5 days with definite abscesses in the various organs, particularly the kidneys. *C. tropicalis* and *C. stellatoidea* fail to kill the animal but cause a definite and characteristic torticollis with abscesses in the brain and healed lesions in the kidneys. They produce gas on glucose and maltose and *C. tropicalis* produces gas on sucrose. Morphologically, they show differences, but also some common characteristics such as the production of chlamydospores. The chlamydospore production of *C. tropicalis* is, however, scanty, only an occasional one being seen. Serologically they are very closely related, showing complete cross agglutination, but slight differences in absorption.

The remaining 5 species show a greater diversity of characteristics than do those in the *C. albicans* group. They are distinct from this group and from each other serologically. They do not produce gas on maltose and show no virulence for the rabbit. Although not formerly considered pathogenic for humans, recent reports show that all 5 have been implicated in human infections.

THERAPY

The numerous methods of therapy available until 1954 have been summarized by Conant and associates[8] and therefore will not be repeated here. Two additional preparations, however, deserve special mention since they have been found effective in the treatment of moniliasis in our dermatologic clinic here and elsewhere. The first of these is the Carbowax-sulfur ointment described by Hopkins, Weld, and Kesten[18]; the second is the new antifungal antibiotic, nystatin (Fungicidin, Mycostatin) produced by *Streptomyces noursei* and described by Hazen and Brown.[17]

The Carbowax-sulfur ointment is prepared by suspending one per cent flowers of sulfur in a melted mixture of 9 parts Carbowax 1500, and 1 part Carbowax

TABLE I. CHARACTERISTICS OF CANDIDA SPECIES

SPECIES	GIANT COLONY	MORPHOLOGY	SUGAR FERMENTATION	AUXANOGRAM OF SUGARS						BROTH	PATHOGENICITY FOR RABBIT
				GLUCOSE	LACTOSE	SACCHAROSE	GALACTOSE	RAFFINOSE	MALTOSE		
1. *C. albicans*	Cream to greenish tinge. Raised, smooth or rough mycelial fringe, air bubbles.	Cells oval 5-6μ. In corn meal a tree. Mycelium with buds at septa forming clusters. Chlamydospores at room temperature.	Acid and gas in glucose and maltose, acid in saccharose.	+	0	+	+	0	+	Incomplete ring and sediment.	+, dies in 4-5 days with 1 cc. of a 1/1,000 intravenously.
2. *C. tropicalis*	Colony similar but more membranous and rough.	Clusters small and more irregular, also chains of buds from many points along hyphae. Buds 3 x 4-6μ in size. Chlamydospores rare.	Acid and gas in glucose, maltose and saccharose.	+	0	+	+	0	+	Pellicle and sediment.	Not fatal but torticollis with reinoculation.
3. *C. stellatoidea*	Colony similar but more membranous and rough.	Similar to *albicans* though more mycelial development. Clusters more irregular. Chlamydospores as in albicans.	Acid and gas in glucose and maltose. O or A in saccharose.	+	0	0	+	0	+	Incomplete ring and sediment.	Not fatal but torticollis with reinoculation.
4. *C. parapsilosis*	Moist cream.	Mycelium fine 1-2μ, readily falls apart. Giant mycelium seen. Buds 3-4 x 4-5μ in chains at distal ends of cells. Buds usually between cells. No chlamydospores.	Acid and gas in glucose.	+	0	+	+	0	+	No surface growth, sediment.	0
5. *C. krusei*	White, dull, flat.	Mycelium readily separates. 7-9μ elongated buds from distal ends of cells. "Whorls." No chlamydospores. No giant mycelium.	Acid and gas in glucose.	+	0	0	0	0	0	Thin film.	0
6. *C. guilliermondii*	Similar to albicans.	Mycelium fine with small clusters. A miniature *albicans* though without chlamydospores.	Acid and gas in glucose and saccharose.	+	0	+	+	+	+	No surface growth.	0
7. *C. pseudotropicalis*	Cream to tan dull growth.	Elongated cells, readily falls apart.	Acid and gas in glucose, saccharose, and lactose.	+	+	+	+	+	0	No surface growth.	0
8. *C. lipolytica*	Yellowish growth, glistening and corrugated.	Pseudo and true mycelium. Blastospores sparce. Verticillate or short chains 2-4.5 x 9.5-22μ.	No gas.	+	0	0	0	0	0	Thin, fragile film.	0

VOL 5 1957

1540; this is applied topically twice daily to infected nails or intertriginous areas, or in the form of suppositories for vaginal moniliasis. This preparation has been found of value by several of the dermatologists in our clinic, except for cases of generalized cutaneous or systemic moniliasis.

The second therapeutic agent, nystatin, has been used successfully in our clinic in adults and children with oral thrush, and in children with cutaneous moniliasis, diaper rash, and vulvovaginitis. All responded rapidly to topical applications of nystatin. The only resistant case encountered so far in our clinic has been a patient with widespread cutaneous and gastrointestinal moniliasis of over 16 years' duration. Even in this patient, the combined use of oral and topical nystatin resulted in elimination of *Candida albicans* and other yeastlike fungi from the gastrointestinal tract and complete clearing of the thrush patches in the oral mucosa. Considerable improvement also occurred in the skin and although the extremely hyperkeratotic nails have persisted, the dramatic improvement in this patient after twelve months of nystatin treatment has been far greater than any obtained with the many forms of therapy previously used in this case.

VOL.
5
1957

Although blood levels of this antibiotic have not been measurable, reports in the literature show that systemic moniliasis responds to oral treatment with nystatin. Cures of pulmonary and urinary tract infections have been reported.[2,27]

In view of the favorable response obtained in our clinic as well as elsewhere,[2,10,12,25,27,28] there is little doubt that nystatin is the antifungal agent of choice for the treatment of all forms of moniliasis. Since the gastrointestinal tract is frequently the reservoir of Candida in the human body, the combined use of oral with topical therapy should be urged even for cases with localized cutaneous lesions. Treatment should be continued for several days following the disappearance of signs of infection. In cases where there is considerable erythema or secretion in the skin lesions, the use of ointment or dusting powder should be preceded by treatment with wet compresses of potassium permanganate, 1:8,000 or silver nitrate, 1:5,000. The only side effects encountered with nystatin have been in cases where excessively high dosage has been employed. These have been diarrhea or cutaneous irritation, both of which disappear with a reduction in dosage.

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HISTOPLASMOSIS

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VOL. 5 1957

HISTOPLASMOSIS is an infectious but noncontagious disease caused by the fungus, *Histoplasma capsulatum*. Man, as well as a variety of wild and domestic animals, are susceptible to invasion by this agent.[1,2] Once thought to be a rare and fatal disease,[3] histoplasmosis is now known to be widespread throughout the United States and parts of Mexico, Central America, South America, and other parts of the world.[4] During the past ten years a voluminous amount of data has been published concerning the epidemiology, clinical aspects, pathogenesis, pathology, laboratory diagnostic methods, and methods of treatment of histoplasmosis. Several reviews of current knowledge of this disease have been published recently and the reader is referred to these[4-7] for more detailed information than can be given here.

EPIDEMIOLOGY AND PREVALENCE

The natural reservoir of the *H. capsulatum* is the soil, where it grows as a saprophyte.[8,9] The available epidemiologic and experimental evidence supports strongly the view that histoplasmosis is an air-borne infection.[5,10,11] There is no evidence of person to person or animal to man spread of this disease. On the other hand, on the basis of the isolation of the histoplasma fungus from soils and air where cases have occurred, it is clear that man acquires the infection as a result of the inhalation of the fungus spores growing in the environment.[9,10,11] Once in the respiratory tract and lungs, the histoplasma organism may provoke a variety of pulmonary infections. Under natural conditions of exposure to the histoplasma fungus, the resulting infections are preponderantly inapparent or subclinical. Only rarely does the fungus spread to other organs to produce a fatal illness.[5,6,7]

During the course of infection, whether apparent or inapparent, man develops skin sensitivity to the Histoplasmin antigen which is prepared from the broth filtrate in which the histoplasma fungus is grown.[12] The Histoplasmin skin test has been extensively employed to determine the prevalence of past or present histoplasma infections in population groups. Recently Edwards and Klaer,[4]

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and Manos, Ferebee, and Kerschbaum[13] have summarized data on the distribution of skin test sensitivity to Histoplasmin and of histoplasmosis in the United States and throughout the world. As can be seen in Fig. 1 the highest prevalence of skin test sensitivity in the United States occurs in the population living in the northeast, central, and south central states. In large areas in the middle west, over 60 per cent of the population react in a positive manner to Histoplasmin. On the basis of positive skin tests alone it is estimated that from 25 to 30 million people in the United States have experienced some form of histoplasma infection.[5]

TABLE I. INCIDENCE OF HISTOPLASMOSIS WITH RESPECT TO AGE AND SEX IN THE UNITED STATES AND FOREIGN COUNTRIES

	WHITE		NEGRO		TOTAL
	MALE	FEMALE	MALE	FEMALE	
United States					
Under 10 years	31	31	2	2	66
Over 10 years	82	23	9	2	116
Total					182
Foreign Countries					
Under 10 years	1	0	3	3	7
Over 10 years	18	0	4	0	22
Total					29

(From Loosli: Med. Clin. North America 39:171-199, 1955.)

Before the use of the skin test in 1945, only the fatal form of histoplasmosis was recognized.[3] The reported cases to date probably represent only a small portion of fatal histoplasmosis and of cases surviving clinical illness. Many etiologically proved cases of fatal histoplasmosis have not been reported. In all probability many recognized and unrecognized cases have died following which post mortem examinations were not done. Likewise, many nonfatal clinical illnesses due to the histoplasma fungus, have gone unrecognized as such.[5] In Fig. 2 are listed the reported fatal and nonfatal cases of histoplasmosis according to the year of onset. It can be seen that from 1906, when Darling first described histoplasmosis in Panama, to 1935 only 15 cases were reported and all were fatal. Since 1935, cases of fatal and nonfatal histoplasmosis have been increasingly recognized. The number of clinically recognized illnesses due to *H. capsulatum* probably represents both an increase in incidence and, more important, an increase in interest in the disease from the standpoint of clinical manifestations, laboratory diagnosis, epidemiology, and pathology.[5]

Fig. 1.—Distribution of U. S. counties according to per cent of young adult residents reacting to Histoplasmin. (From Manos, N. E., Ferebee, S. H., and Kerschbaum, W. F.: Geographic Variation in the Prevalence of Histoplasmin Sensitivity, Dis. Chest **29**:649-668, 1956, Map 4.)

In Fig. 3 is shown the age distribution of known or reported fatal and nonfatal cases of histoplasmosis from the United States and other countries tabulated according to age at onset of illness. It is of interest that of the fatal illnesses approximately one-fourth have occurred in infants under 1 year of age. The lowest incidence with respect to age is in the 15- to 19-year age group. In the age group from 35 to 65 the number of fatal and nonfatal histoplasmosis

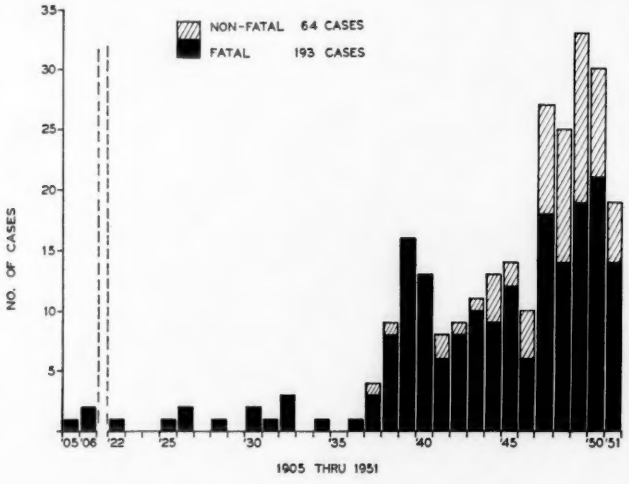

Fig. 2.—Known or reported fatal and nonfatal cases of histoplasmosis from the United States and other countries tabulated according to year of onset of illness. (From Loosli, C. G.: Histoplasmosis. Some Clinical, Epidemiological and Laboratory Aspects, M. Clin. North America 39:171, 1955.)

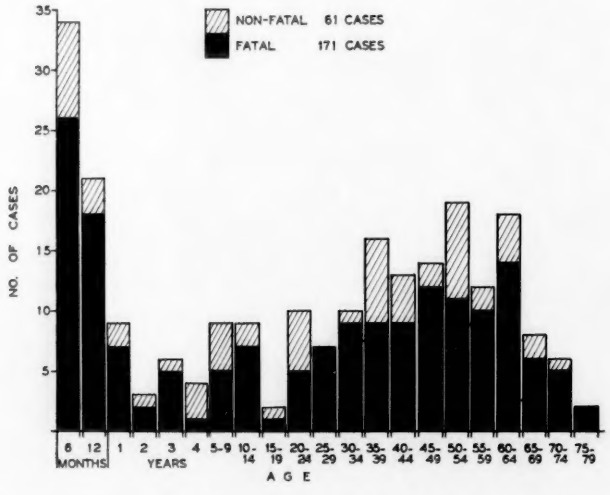

Fig. 3.—Age distribution of known or reported fatal and nonfatal cases of histoplasmosis from the United States and other countries tabulated according to age at onset of illness. (From Loosli: M. Clin. North America 39:171, 1955.)

cases is approximately the same. As seen in Table I there is no sex difference in frequency of clinical histoplasmosis in patients under 10 years of age while in individuals over 10 years of age infections have been reported 4 times more frequently in males than in females.[5,14]

Although several epidemic outbreaks of nonfatal pulmonary histoplasmosis have occurred in areas where the skin test prevalence of histoplasmosis is moderate, most reported cases of fatal and nonfatal Histoplasmosis occurs in areas where Histoplasmin skin test sensitivity is highest.[5,15] This is shown in Table II. From the 10 states, shown in Fig. 1, which have a skin test sensitivity prevalence of 40 per cent or higher, 63 per cent of the known cases of histoplasmosis have been reported.[5] The marked geographic differences in prevalence of skin test sensitivity and origin of reported cases strongly suggests, as Manos and his associates[13] point out, that socioeconomic factors such as crowding, occupation, patterns of living, etc., do not play an important role in the epidemiology of histoplasmosis. The important factor is geography. Edwards and Klaer[4] state: "Where a man lives rather than with whom he lives is of major importance in determining his chances of becoming infected with the fungus, *H. capsulatum*."

OL.
5
957

TABLE II. THE RELATIONSHIP BETWEEN THE PREVALENCE OF HISTOPLASMIN SENSITIVITY IN THE POPULATION AND 277 KNOWN CASES OF HISTOPLASMOSIS

HISTOPLASMIN SENSITIVITY (PER CENT)	NUMBER OF STATES*	TOTAL POPULATION (MILLION)	KNOWN CASES	
			NUMBER	PER CENT
0–19	27	78	25	11
20–39	12	35	59	26
40–90	10	37	143	63

(From Loosli, C. G.: M. Clin. North America 39:171-199, 1955.)
*Includes the District of Columbia.

PATHOLOGY

The pathogenesis and pathology of histoplasmosis have been described in numerous case reports and reviews.[3,7,14,16] It should be recalled, however, that the fungus, like most others, is dimorphic. When the conidia (spores) are introduced into a susceptible host they are soon converted into yeastlike organisms which are parasitized by reticuloendothelial cells, macrophages, and cells of the peripheral blood.[5,7] Once in a cell they increase by budding. The type of response in man depends on the immune status of the individual. Beamer and his associates[17] describe several types of tissue sections. In infants, whose immune response is poor, little or no inflammatory reaction is observed. The invading fungus causes proliferation of the histiocytes to form granulomas without caseation; there results marked enlargement of the liver, spleen, and lymph nodes where these cells are abundant. In some lesions there is fibroblastic proliferation at the periphery. In older persons the granulomatous lesions may be localized or widely disseminated but show more inflammatory response at the periphery

with caseation in the central portion as seen in the reinfection type of tuberculosis. In the lung, necrosis and caseation may be so extensive, as in tuberculosis, that cavity formation occurs.[5,7,14,16,18]

In young individuals dying of disseminated histoplasmosis, organisms may invade every tissue and organ of the body. In 39 infants under 4 years of age coming to autopsy, Beamer and his associates[17] found the liver, lymph nodes, lung, spleen, bone marrow, kidney, and ileum significantly more frequently involved than in an older group of 84 patients. In the older group the lung was the most frequently involved organ. Likewise, lesions of the tongue, nasopharynx, and larynx were almost exclusively present in the older group.[17] Why histoplasmosis disseminates in some cases and not in others is not known. Dosage, virulence of the organism, age, and immune state of host probably all play a part. Although histoplasmosis is occasionally observed in association with tuberculosis, carcinoma, Hodgkin's disease, and sarcoidosis this is only a casual finding.

VOL 5 195

CLINICAL AND PHYSICAL FINDINGS

Histoplasmosis is a ubiquitous disease. Its clinical manifestations are many and nonspecific.[5,11,20,21,22] Smith[19] lists 4 clinical types. They are (a) the primary pulmonary, (b) the primary mucocutaneous, (c) the primary disseminated, and (d) the reinfection pulmonary types. All 4 types occur, most likely, from inhalation of fungus spores for the available evidence points to the respiratory tract as the only portal of entry of *H. capsulatum* regardless of the resulting clinical manifestations.[5] Transitory dissemination of the fungus with skin and mucous membrane lesions has been observed during the course of acute nonfatal pulmonary histoplasmosis acquired in the laboratory. Healing primary pulmonary foci have been described in patients dying of disseminated illnesses.[7,14,17] The clinical and physical findings in histoplasmosis vary widely depending on degree of exposure, age, and immune state of the individual, the site or sites of involvement, severity, and duration of illness.[5,7,20,21,22]

Since histoplasmosis is primarily an air-borne disease, the pulmonary system is most frequently involved. In clinically apparent infections symptoms may be mild or severe and begin gradually or abruptly from 10 days to 3 weeks after inhalation of the fungus. There is fever varying from 101° to 103° F. associated with sweating, malaise, myalgia, arthralgia, and headache. Cough with or without sputum may be the only symptom referable to the respiratory tract. Rarely is there pleural pain or effusion.[5,7,10]

In the acute phase of pulmonary histoplasmosis, physical findings in the chest may be absent or minimal even when the lesions are widespread. Pulmonary histoplasmosis may occur without showing abnormal signs by x-ray. When there is x-ray evidence of a histoplasma infection of the lung, it may be represented as hilar lymphadenopathy, small or large single lesions; or small or large multiple noncalcified infiltrates throughout the lung fields. The varied radiologic features of pulmonary histoplasmosis have been recently reviewed and adequately illustrated.[5,7,23,24]

In acute pulmonary histoplasmosis regardless of severity the total white blood cells are only slightly increased and there is no shift in the differential

count.[10] In disseminated illnesses there is usually leukopenia and moderate anemia. The red blood cell sedimentation rate is usually moderately elevated both in acute and chronic illnesses due to *H. capsulatum*. The duration of acute pulmonary histoplasmosis may vary from one week to three months or longer. Recovery is the rule and when symptoms subside a return to a state of physical well being occurs quite rapidly although the pulmonary infiltrates may persist for several years before becoming calcified.[10] In some individuals the infiltrates may persist indefinitely as noncalcified focal "coin" lesions. With recovery pulmonary histoplasma lesions of the single and miliary variety have been observed to undergo resolution.[24]

During the past several years chronic active pulmonary histoplasma infections with cavitation have been recognized with increasing frequency.[25,26,27] The symptoms are those of gradual weight loss, mild fever, and a productive cough yielding sputum from which the histoplasma fungus can be isolated readily. The pulmonary lesions cannot be distinguished from those caused by the tubercle bacillus.[24,26,27] Thus, many of these cases are admitted to and found in tuberculosis sanatoriums. Furcolow and Brasher[27] estimate that, if an adequate search were made, "approximately 400 mycologically proved" cases of histoplasmosis might be found in these sanatoriums in areas where the Histoplasmin sensitivity exceeds 50 per cent.

OL.
5
957

DIFFERENTIAL DIAGNOSIS

Pulmonary histoplasmosis must be considered in the differential diagnosis of many acute and chronic infections, including acute respiratory diseases due to viral and bacterial agents, viral pneumonia, other mycotic infections, tuberculosis, and fevers of unknown orgin. Healed but not yet calcified histoplasma granulomas may simulate primary or metastatic neoplasms, lymphomas and sarcoidosis. Miliary calcification of the lungs is now considered generally to represent a healed histoplasma infection rather than healed tuberculosis.

Even though the portal of entry is the respiratory tract, many other organs besides the lungs may become the focus of infection with *H. capsulatum*. In disseminated infections, especially in infants, where there is enlargement of the liver, spleen, and lymph nodes, leukemia is often initially considered. Meningitis has also been shown to be caused by histoplasma fungus. This agent has been found also to be the cause of skin ulcerations and mucous membrane lesions of the mouth, tongue, pharynx, larynx, intestine, penis, and anus which, at first, were almost invariably considered to be cancerous. Histoplasmosis has also been described in association with tuberculosis, Hodgkin's disease, and sarcoidosis.[5,7]

DIAGNOSTIC PROCEDURES

In geographic areas where Histoplasmin sensitivity is high, all varieties of histoplasma infections are becoming more frequently recognized.[5,7] By maintaining a high index of suspicion that disease may be due to *H. capsulatum*, one is able in the early phase of illness to utilize more effectively laboratory diagnostic procedures. These include (a) skin tests, (b) serologic determination of specific antibodies, (c) methods for isolation of fungi, and (d) study of biopsy material.

Skin Test.—The Histoplasmin skin test is now widely used both as an epidemiologic tool and as an aid in diagnosing histoplasma infection.[4,12,13,28] Skin test sensitivity develops during the third or fourth week after onset of infection. Conversion of a negative reaction with Histoplasmin to a positive one is good evidence that the etiologic agent is *H. capsulatum*. Often, however, the skin test is not applied until after sensitivity develops. Following recovery, Histoplasmin sensitivity persists for many years and may be the only evidence of previous exposure to the fungus.

A positive Histoplasmin skin test has the same significance in histoplasmosis as a positive tuberculin test in relation to tuberculosis.[28] It signifies only sufficient exposure to the histoplasma fungus to have elicited sensitivity. A small number of individuals reacting strongly to Histoplasmin may occasionally react, but to a lesser degree, to Blastomycin and Coccidioidin. This phenomenon, however, should not invalidate the Histoplasmin skin test as an epidemiologic tool or as an aid in the diagnosis of histoplasmosis.[28] When used as diagnostic procedures all 4 standardized antigens*—tuberculin, Histoplasmin, Coccidioidin, and Blastomycin—should be applied simultaneously.[29] The one eliciting the greatest reaction is then interpreted as indicating the most probable etiologic agent. In fatal disseminated histoplasmosis the skin test may be negative in the terminal stage of the disease. There may be also a temporary reversion from a positive to a negative reaction during other severe illnesses. Histoplasma infections do not provoke cross sensitivity to old tuberculin or purified protein derivative (PPD).[28,29]

Complement Fixation Test.—Because it is often not possible to isolate *H. capsulatum* in cases of acute pulmonary histoplasmosis, serologic diagnostic procedures are most important. Complement fixing, precipitin and collodion agglutinating antibodies appear in the blood during the third or fourth week of a histoplasma infection. The complement fixation test (C. F.) employing heat-killed, whole-yeast phase organisms as antigen is most widely employed. Histoplasmin also has been used as antigen in the C. F. procedure.[30,31] The persistence and level of C. F. antibody depends on the severity and chronicity of infection. C. F. antibody to the *H. capsulatum* antigen usually can be demonstrated for several weeks to months after symptoms have subsided. After recovery from a histoplasma infection the C. F. antibody titer gradually falls and eventually can no longer be demonstrated.[5,10,30,31] In individuals with chronic, active pulmonary infections with cavitation C. F. antibodies may persist at a high level for several years.[5,25] Examples of C. F. antibody titers which illustrate the above points in individuals with acute and chronic pulmonary infections are shown in Table III.†

Because the C. F. test with the whole-yeast antigen eventually becomes negative when recovery from histoplasmosis is complete, any demonstrable anti-

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*Histoplasmin, Blastomycin, and tuberculin PPD (purified protein derivative) are made for commerical distribution by the Parke Davis Company. Cutter Laboratories make Coccidioidin and the Sharp and Dohme Company also makes PPD. Directions for application are supplied by the distributors.

†I am indebted to my associates, Dr. John J. Procknow and Dr. D. F. Loewen, for permission to use data on the C. F. test from a cooperative long-term study.

TABLE III. COMPLEMENT FIXATION ANTIBODY TITERS TO HISTOPLASMA WHOLE YEAST ANTIGEN IN CASES OF ACUTE, SUBACUTE, AND CHRONICALLY ACTIVE PULMONARY HISTOPLASMOSIS

PATIENT	DURATION OF ILLNESS			TIME FOLLOWING FIRST VISIT (MONTHS OR YEARS)						
	PRIOR TO FIRST VISIT	TOTAL	<1	1	2	3	4	6	8	12
Acute Histoplasmosis (Months)										
R. D.	2 wk.	5 wk.	Neg.	1:32	1:16	1:4	Neg.	Neg.	—	Neg.
M. P.	10 days	8 wk.	Neg.	1:32	1:16	1:4	Neg.	Neg.	Neg.	Neg.
J. J. P.	2 wk.	8 wk.	Neg.	1:16	1:8	1:2	Neg.	Neg.	Neg.	Neg.
F. T.	2 wk.	8 wk.	Neg.	1:20	1:10	1:5	—	—	Neg.	Neg.
*L. O. J.	2 wk.	7 wk.	Neg.	1:8	1:16	1:32	1:8	1:2	1:2	1:2
Subacute Histoplasmosis (Months)										
M. K.	6 wk.	Not ill	—	1:20	1:40	—	1:5	1:5	Neg.	—
P. D.	5 wk.	6 wk.	—	1:20	1:20	1:10	1:10	—	Neg.	—
A. E., Jr.	6 wk.	3 wk.	—	1:160	1:80	1:40	1:20	1:20	Neg.	Neg.
E. E.	6 wk.	3 wk.	—	1:320	1:160	1:80	1:40	Neg.	Neg.	Neg.
A. E., Sr.	6 wk.	12 wk.	—	1:160	1:5	Neg.	Neg.	—	Neg.	Neg.
Chronically Active Pulmonary Histoplasmosis (Years)										
M. H.	9 mo.	Continued	1:64	1:64	1:64	1:32	1:32	—	—	—
J. B. C.	1½ yr.	Continued	—	1:8	1:256	—	1:64	1:64	—	—
J. A. McK.	1½ yr.	Continued	—	1:32	—	1:128	1:64	1:64	—	—
W. T. M.	2½ yr.	Continued	—	1:40	—	1:64	1:64	1:64	—	—
H. G. D.	2½ yr.	Continued	—	1:64	—	1:64	1:64	1:64	—	—

All cases were skin test positive to Histoplasmin, negative to Blastomycin. All but R.D. had evidence of pulmonary involvement. A. E., Sr., and five chronic cases all had positive sputum for *H. capsulatum*, negative for tubercle bacilli. M. P., A. E., Sr., and L. O. J. had positive cultures of blood or bone marrow.

*Titer remains 1:2 at 29 months after onset.

body titer (even as low as 1:2) in an adequately controlled test has significance. When C. F. antibody of any titer is present further tests should be carried out on additional serum samples. A rising titer indicates an acute active infection, while falling titers with subsidence of symptoms indicate recovery. A persistence of antibody titer above 1:8 most likely means continued activity of the histoplasma infection. Titers of 1:2 and 1:4, however, may persist for many months after complete clinical recovery.[5,10] This is also shown in Table III.

Histoplasma capsulatum occasionally provokes circulating antibodies which cross with other fungal antigens, particularly *Blastomyces dermatitidis*, and, less frequently, with *Coccidioides immitis*.[31,32,33] The C. F. antibody titer in a given serum, however, is usually significantly higher with the homologous than with

the heterologous standardized antigen. This phenomenon of cross reactions does not invalidate the C. F. test as a valuable diagnostic and prognostic procedure in acute and chronic pulmonary histoplasmosis. Smith[29] recommends the simultaneous use of antigens* prepared from *H. capsulatum* and blastomyces organisms in the C. F. test. During the terminal stage of fatal disseminated illness the C. F. test may be negative. Likewise, patients with skin ulcers or mucous membrane lesions due to *H. capsulatum* may or may not show C. F. antibodies.[5,7]

Biopsy.—As a result of careful examination of biopsy material, *H. capsulatum* has been found to be the unsuspected etiologic agent in a wide variety of lesions. A microscopic diagnosis depends on observing yeast-phase organisms in the cytoplasm of reticuloendothelial cells, macrophages, and peripheral blood cells (Fig. 4). Ante mortem diagnosis of generalized histoplasmosis is made by examining tissue specimens of blood, bone marrow, spleen, liver, lymph nodes, and adrenal glands for the presence of intracellular organisms.[5,7,34] Particularly, biopsy specimens of ulcerative lesions suspected of being cancer of the skin, buccal cavity, and anus have shown the agent to be *H. capsulatum.*[7,14] Biopsies of granulomatous lesions and lymph nodes have yielded a diagnosis of histoplasmosis when tuberculosis, lymphoma, sarcoidosis, or Hodgkin's disease was initially suspected. In pulmonary histoplasmosis, where there is an abundance of sputum, macrophages filled with intracellular organisms may be observed in sputum smears.[5,24]

At autopsy in cases showing systemic involvement, intracellular organisms such as shown in Fig. 5 are usually seen in organs rich in macrophages and reticuloendothelial cells.[5,7,34] In well-fixed material the organisms stain a deep purple with the hematoxylin-eosin technique. In poorly fixed or caseous material they may take the stain poorly or not at all.[34] In healing granulomatous lesions where only scattered cells contain organisms, the Hotchkiss McManus stain is of value in identifying them.[35] This and the Gridley stain[36] have also proved of great value in demonstrating the chitinous "ghost" remains of dead or dying histoplasma organisms in caseous material in granulomatous "coin" lesions (Fig. 6).[5,16,18,37] Emmons calls attention to the fact that a histopathologic diagnosis is not infallible, for yeast forms of other fungi may occasionally "mimic the morphology and intracellular habit of histoplasma."[6]

Culture.—A definitive diagnosis of histoplasmosis can be made only by isolation of the fungus.[38-41] *H. capsulatum* is not fastidious and will grow on a variety of media. Ajello[38] outlines the directions for careful handling of specimens and the preparation of different media employed for the culture of fungi including *H. capsulatum.* For routine diagnostic purposes enriched blood agar to which penicillin (20 units per milliliter) and streptomycin (40 units per milliliter) are added, Sabouraud's medium, and corn-meal agar are usually employed.[39] Sputum, bronchial secretions, exudates, fluid sediment, blood, bone marrow, ground biopsy material (lymph node, liver, spleen, adrenal, mucous membrane lesions, etc.) are streaked directly on the surface of previously prepared media.[38-41] In our experience, blood agar incubated at room temperature has yielded the great-

*Histoplasmin and Blastomycin for C. F. antigen supplied by Parke Davis and Company.

est number of positive cultures.[5,10] Cultures for fungi in test tubes or Petri plates should be sealed to prevent drying and observed for 6 to 8 weeks before being discarded as negative. If repeated cultures of the blood and bone marrow during the febrile stage of acute pulmonary histoplasmosis were routinely done,

VOL.
5
1957

Fig. 4.

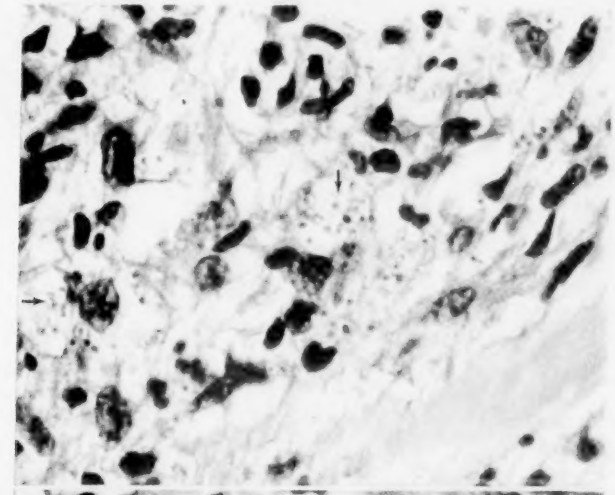

Fig. 5.

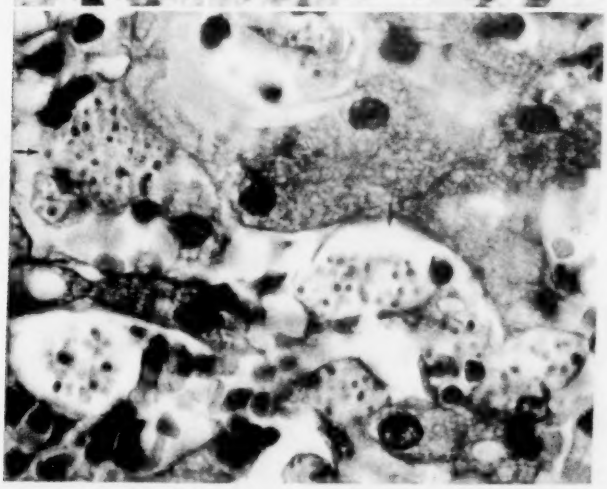

Fig. 4.—Photomicrograph of adrenal gland biopsy from a 51-year-old woman (F. M.) who had prolonged fever and symptoms of Addison's disease, showing intracellular yeast-phase histoplasma organisms (arrows). Skin tests positive with Histoplasmin and negative with old tuberculin. C. F. antibody titer repeatedly present in serum dilution of 1:10. The patient eventually died of disseminated histoplasmosis with a small focal lesion demonstrated in the lung at autopsy. Hematoxylin-eosin stain. (×1,400; reduced ⅓.)

Fig. 5.—Photomicrograph of liver of a 6-month-old infant (B. C.) who died of generalized histoplasmosis. Note histiocytes filled with histoplasma yeast cells and absence of inflammatory reaction. Hematoxylin-eosin stain. (×1,100; reduced ⅓.)

frequent transient dissemination of the fungus would probably be revealed. In three such cases where this was done *H. capsulatum* was isolated from the blood or bone marrow or from both 3 to 6 weeks after onset; all recovered without benefit of specific therapy.[5]

H. capsulatum growing on blood agar at room temperature exhibits a variety of colony forms. The usual one is waxy, grayish-brown, cerebriform in shape, and makes its appearance after 2 to 3 weeks of incubation (Fig. 7).[5,10,24] On blood agar grown at 37° C. it grows in the yeast phase as mucoid moist colonies. On Sabouraud's and corn-meal agar *H. capsulatum* grows as white fluffy mycelial colonies (Fig. 8). Micro- and macroconidia (spores) characteristic of *H. capsulatum* are identified by examining the mycelial culture under low-power magnification (Fig. 9). Histoplasma organisms grown on blood agar must be transferred to Sabouraud's or corn-meal media for identification of the fungus.[5,10,24]

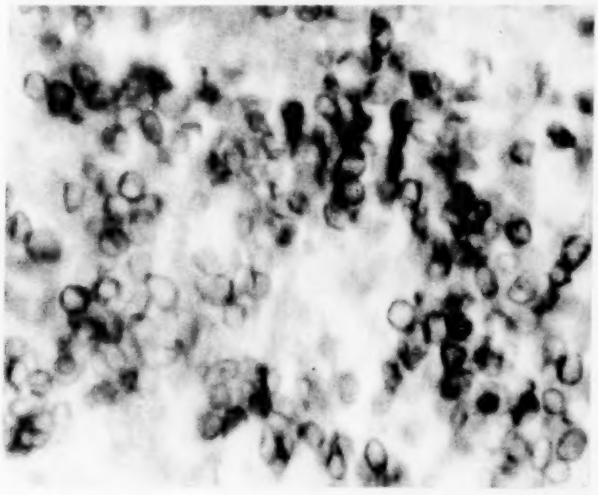

Fig. 6.—"Ghosts" of yeast cells in caseous material in center of solitary pulmonary histoplasmoma as revealed by the Gridley stain. (×2,400; reduced ⅓.)

Animal Inoculation.—The mouse, guinea pig, and hamster are used in the isolation of *H. capsulatum*. The white mouse, which is highly susceptible, is most frequently employed, and is essential for the isolation of *H. capsulatum* from the soil.[8,42] Suspected material which is prepared for direct culture should also be inoculated intraperitoneally in mice. The supernate of a soil suspension after the addition of penicillin (50 units per milliliter) and streptomycin (100 units per milliliter) is also inoculated intraperitoneally. After 6 to 8 weeks the animals are sacrificed. The pooled livers and spleens are minced in antibiotic broth, spread liberally on blood agar and Sabouraud's or corn-meal agar and incubated at room temperature for 6 to 8 weeks, care being taken to seal the cultures to prevent drying. The morphologic appearance of fungus isolates from the mouse is the same as that seen on direct plating on the above media and as shown in Figs. 7 and 8.

VOL 5 195

OL.
5
957

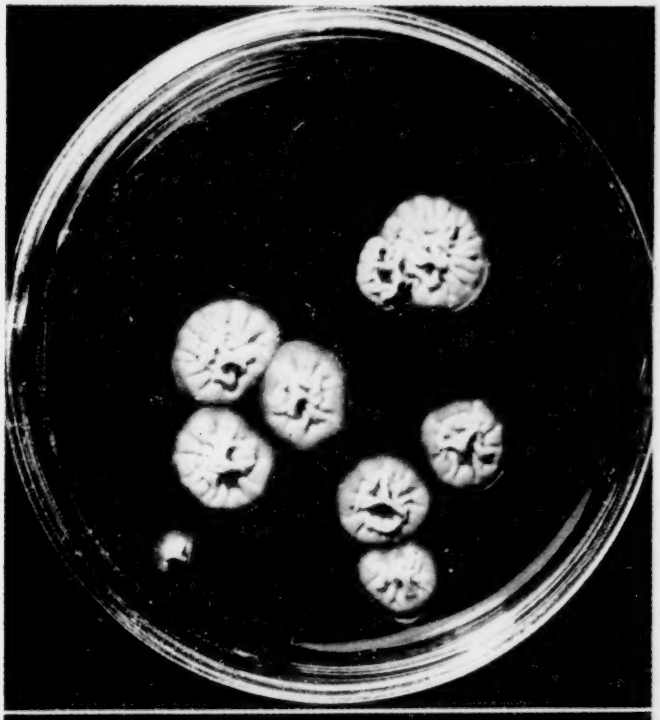

Fig. 7.

Fig. 8.

Fig. 7.—Direct culture of sputum on enriched blood agar at room temperature showing grayish brown and characteristically cerebriform histoplasma colonies. Patient had chronic active pulmonary histoplasmosis with bilateral cavitation.

Fig. 8.—Direct culture of sputum on corn-meal agar at room temperature showing white mycelial growth of *H. capsulatum*. Sputum from same patient as Fig. 7.

TREATMENT

There is, as yet, no specific therapy for histoplasmosis.[5,7,19,43] Fortunately, the great majority of patients with this disease recover. Only the rare disseminated infection terminates fatally, for an increasing number of cases of acute and chronic histoplasmosis with evidence of systemic involvement by positive culture of the blood or bone marrow have survived.[5,7,17,20] This fact, as well as the extreme variation in the clinical course of the disease, should be kept clearly in mind in the evaluation of any therapeutic agent for the treatment of histoplasmosis. As Silverman and his associates[7] state, "supportive treatment in the form of dietary measures, food supplements, transfusions, effective treatment for underlying or superimposed disease, is governed by considerations unrelated to

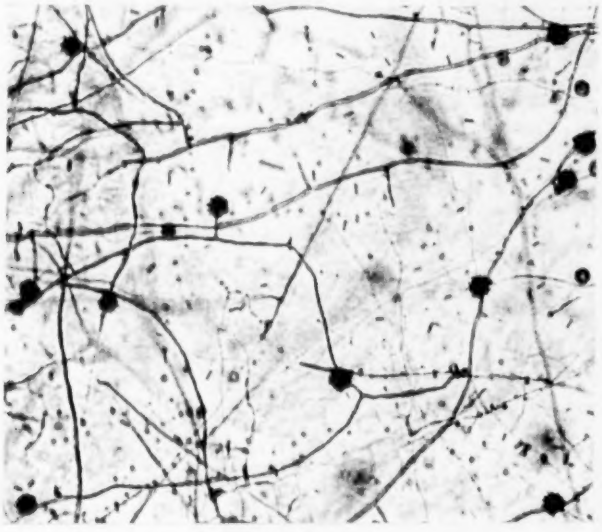

Fig. 9.—High-power photomicrograph of histoplasma mycelial phase culture showing macroconidia with characteristic tuberculate borders. Unstained. (×300; reduced ⅓.)

the presence of histoplasmosis and the indications are well known and clearly defined." Loewen[25] has followed for several years a number of cases of active pulmonary histoplasmosis with cavitation and observed marked clinical improvement on bed rest in the sanatorium. Some of these patients had antituberculosis therapy.

A wide variety of chemotherapeutic and antibiotic agents have been employed in the treatment of histoplasmosis without success.[5,7,19,43,44] The sulfonamides and antibiotics, so successful in the treatment of bacterial infections in man, are ineffective against histoplasmosis. Likewise, antibiotics and chemotherapeutic agents effective in the treatment of other fungus infections are also ineffective in the treatment of this disease.[5,7,19,43,44] There are a number of fungistatic and fungicidal agents which have exerted a favorable response in the treatment of experimental histoplasma infections, as reported by several investigators at a recent symposium on the treatment of fungus infections.[44] Those

which have been or are now under clinical investigation and deserve further study are Mycostatin (Squibb), Amphotericin (Squibb), ethyl vanillate (Squibb), MRD-112 (Merrell), and 2-hydroxystilbamidine (Merrell). Basic information on the use of these drugs may be obtained from the appropriate pharmaceutical company.

Cases of histoplasmosis suitable for evaluation of therapeutic agents include those with (a) skin or mucous membrane lesions, (b) chronic active pulmonary histoplasmosis with cavitation, or (c) cultural evidence of disseminated illness. Failure of some of the agents listed above to effect favorably the course of pulmonary cavitary histoplasmosis may have been due to the small amount given and the short duration of treatment.[27,45] Because of the intracellular nature of histoplasmosis, the amount of drug or antibiotic should be gradually increased to the maximum tolerated dose and given over a period of several weeks to months.

In selected cases of active pulmonary histoplasmosis surgical removal has been successful.[5,27,46] Many granulomatous "coin" lesions of the lungs, the residual of histoplasma infections, have been removed successfully by segmental resection.[16,18,37]

OL.
5
957

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VOL.
5
1957

EPIDEMIC HISTOPLASMOSIS

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AS THE result of extensive skin testing surveys by Christie, Palmer, Furcolow and Loosli, it has been estimated that probably 30 million persons in central United States have been infected with the fungus *Histoplasma capsulatum*.[21] Compared with the over-all incidence of the disease the number of clinical cases of epidemic histoplasmosis is numerically quite unimpressive. However, it is from this small group that much of our present knowledge of the epidemiology and the nature of *Histoplasma* infections has been uncovered.

Only a little more than a decade ago a series of reports appeared in the literature describing an obscure pulmonary disease of epidemic nature. The occurrence of miliary pneumonitis following a number of unusual activities led to such colorful and descriptive names as "acute miliary pneumonitis," "primary atypical pneumonia," "an unusual pulmonary disease," "pneumonitis of unknown etiology," "cave sickness," and "an unusual type of pulmonary disease." Furcolow and Grayston in 1952[5,6] reviewed the characteristics of 13 such epidemics involving 151 persons. This included retrospective studies on these previously described unusual outbreaks. Based on the results of skin tests, serologic studies, the presence of miliary pulmonary calcifications and the isolation of *H. capsulatum* at the "point sources" of infection, these workers concluded that *H. capsulatum* was the etiologic agent. Loosli in 1955[21] briefly discussed the problem of epidemic histoplasmosis and located 21 different outbreaks. Since this report several additional reports have appeared in the literature describing single outbreaks. In addition we have investigated a number of epidemics which have not yet been reported. It is the purpose of this paper to review briefly all of these epidemics and the problem in general.

MATERIALS AND METHODS

The epidemics of histoplasmosis were uncovered by correspondence with persons who reported instances of unusual pulmonary disease, or when physicians referred cases to us for consultation. The criterion for an epidemic is that two or more persons acquire their infection at the same "point source."

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The criteria used to establish *H. capsulatum* as the etiologic agent in an outbreak of pulmonary disease are those outlined previously.[5,6] These are: (1) the presence of positive Histoplasmin skin tests; (2) the presence of positive serologic tests for histoplamosis; (3) the isolation of *H. capsulatum* from the "point source"; (4) the development of miliary calcification in the x-rays of the lungs (this is not apparent until months or years after the episode); (5) (in some epidemics) the isolation of the fungus directly from one or more patients.

RESULTS

To date we have information on 41 known epidemics of histoplasmosis. This information is summarized in Table I and Fig. 1. Thirty-eight of these have occurred in rather widely separated areas in 17 states of continental United States, and have involved over 400 persons. The largest of these epidemics, which involved 146 school children in Mountain Home, Arkansas,[22] has recently been described by our group.

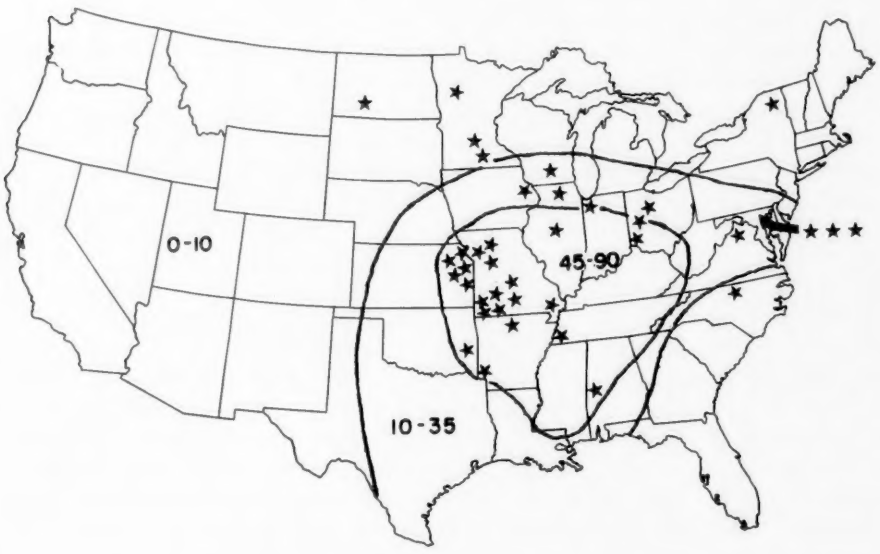

Fig. 1.—Location of epidemics of histoplasmosis in relation to prevalence of Histoplasmin sensitivity.

Recently, outbreaks of "cave sickness" have been recognized in foreign countries. At least four separate epidemics have occurred in South Africa and are thought to be associated with bat guano in caves where *H. capsulatum* has been isolated from the air by Murray.[29] Three were from near Johannesburg, South Africa, and others were from Northern Transvaal and Southern Rhodesia. Conversion of the skin tests and positive serologic tests were demonstrated in the Johannesburg cases.

Another outbreak is the much published "Tingo Maria fever"[28] of Peru. In the Tingo Maria cave, *H. capsulatum* was isolated from the soil enriched by the droppings of the cave-dwelling oil bird (*Steatornis caripensis*), a rare bird whose rich oil is used as a substitute for butter by the Peruvians. Many casual infections of tourists were reported and in October, 1955, a small epidemic (involving 6 persons) occurred. Positive serology and skin tests, and isolation of the organism from the soil were demonstrated. Recently another epidemic has been reported from Venezuela.[30]

A summary of the pertinent information on the 41 epidemics is seen in Table I. These epidemics will be discussed from the clinical and x-ray viewpoint and the serologic, epidemiologic, and reservoir studies will be summarized.

Clinical Features.—Grayston and Furcolow[5,6] in a review of almost 200 cases of disseminated pulmonary infiltration found a remarkably constant clinical picture. The onset of symptoms is usually sudden and characterized by generalized malaise followed in about 24 hours by chills, fever, and nonproductive cough. Remitting temperatures to 106° F. are not unusual, and a low-grade temperature may persist for many weeks. Many of these patients complain of chest pain, which may be one of two types. The first is a retrosternal pressing sensation that is aggravated by deep inspiration and swallowing, and is probably related to expanding mediastinal lymph nodes. The second is pleuritic pain which is not uncommon and usually of short duration. Small pleural effusions from which *H. capsulatum* has been isolated have been demonstrated in a few cases. Dyspnea is not uncommon in the most severe cases but usually lasts for only a few days with return to apparently normal functional capacity. During the first month weight loss is pronounced, and is probably related to the increased metabolic demands resulting from fever. With subsidence of fever the patient usually feels well except for rather marked fatigue, which may persist as long as six months. Despite the marked changes on x-ray there is a dearth of physical findings. Routine laboratory examinations are usually within normal limits except for an elevated sedimentation rate.

One of the most important facts brought out by this follow-up study of epidemics is that many small family epidemics occur which may include younger children. This changes basically our concept of epidemic histoplasmosis, and raises the question of the relationship of epidemic histoplasmosis to the endemic disease. It is quite likely that the recognition of epidemic histoplasmosis in other age groups and in smaller groups is simply due to attention aroused by the coincidence of lesions rather than by the severity of the disease. The illness spectrum in the epidemics is extremely variable and runs from asymptomatic illness, (sometimes with positive chest x-ray) to severe and sometimes fatal illness in other members of the same group. Indeed the available evidence seems to point to the fact that the spectrum of illness in epidemic histoplasmosis is similar to that of the endemically occurring disease. It is true, however, that probably 60 to 70 per cent of endemic cases are asymptomatic while the majority of epidemic cases have clinically recognizable illness. The occurrence of illness of varying severity among members of a single outbreak can usually be correlated with the degree of exposure. The fact that the majority of cases of epidemic histoplasmosis have rather severe clinical disease and marked x-ray changes can

VOL.
5
1957

TABLE I. SUMMARY OF EPIDEMICS OF HISTOPLASMOSIS

LOCATION, YEAR, AND REFERENCE NUMBER	NUMBER ILL	SOURCES	HISTOPLASMOSIS FROM SOIL	HISTOPLASMIN SKIN TEST (NUMBER POSITIVE)	HISTOPLASMOSIS SEROLOGIC TESTS (NUMBER POSITIVE)
Plattsburg, N. Y., 1938[1,2]	23	Schoolhouse; shoveling pigeon manure	—	13/13	8/9 after 4 years
Camp Crowder, Mo., 1943[3]	40	Farm Buildings; demolition and cleaning	+	All tested (incomplete)	(incomplete)
Camp Gruber, Okla. 1944[4]	35	Storm cellar; built fire	+	22/22	Most sera positive 4-21 after 7 years
Topeka, Kansas 1944[5,6]	3	Old barn	+	3/3	Not done
Williamsburg, Kans. 1946[5,6]	5	Farm	—	5/5	0/3 after 18 months
Kansas City, Mo., 1947[5,6]	5	Storm cellar	—	5/5	5/5
Foreman, Arkansas 1947[7]	25	Cave excavating	+	12/13	10/12 acute 4/13 after 4 years
Cincinnati, Ohio 1947[8]	12	Tower; shoveling pigeon manure	+	All tested	10/11 acute
Warrenton, N. C. 1947[9]	7	Church belfry; pigeon manure	—	6/6	4/4 acute
Detroit Lakes, Minn., 1948[10]	6	Basement of farm house	+	6/6	4/6 after 4 years
Madison, Wisconsin, 1948[11]	10	Woods; digging worms	+	10/10	9/10 acute
Taneytown, Md., . 1948[12]	4	Barn manure	—	4/4	4/4
Maple Lake, Minn., 1949-1953[13,14]	6 2	Farm	— —	6/6 1/2	2/6 (4 years) 2/2
Lowell, Ind., 1950[15]	6	Silo; shoveling sawdust material	+	6/6	5/6 acute
Bellevue, Iowa, 1951[5,6]	4	Farm yard	+	4/4	2/3
Havana, Ill. 1951[5,6]	2	Hollow tree	+	2/2	2/2
Calvert Co., Md., 1951[16]	2	Scraping bridges; bat dung	—	2/2	1 positive 1 doubtful
Mandon, N. D. 1952[5,6]	5	Schoolhouse; pigeon and bat manure	—	5/5	4 positive 1 doubtful
Atchinson, Kans., 1952[17]	5	Chicken house	+	5/5	5/5
Wheaton, Md., 1952[18]	4	Cleaning cellar	—	4/4	4/4

TABLE I—Continued

LOCATION, YEAR, AND REFERENCE NUMBER	NUMBER ILL	SOURCES	HISTOPLASMOSIS FROM SOIL	HISTOPLASMIN SKIN TEST (NUMBER POSITIVE)	HISTOPLASMOSIS SEROLOGIC TESTS (NUMBER POSITIVE)
Pisgah, Ohio, 1952[19]	2	Farm	—		4/4 acute
Memphis, Tenn., 1952[20]	2	Chicken manure in garden soil	+	2/2	1/2
Fontana, Kansas 1952*	4	?Farm	—	4/4	1/4 after 2 years
Parkville, Mo., 1953*	4	Attic cleaning; chicken manure	+	4/4	2/4
Lawrence, Kansas 1954*	2	?Barn	—	2/2	2/2
Holt, Mo., 1954*	3	Chicken house	—	3/3	3/3
Macks Creek, Mo., 1954*	2	Chicken house	+	2/2	2/2
Rockford, Ill., 1954[21]	3	Chicken compost heap	+	2/2	3/3
Barry Co., Mo., 1954*	2	?Chicken house	—	2/2	2/2
Stockton, Mo., 1955*	3	Chicken house	+	3/3	2/3
Mountain Home, Ark., 1955[22]	146	?	—	146/146	100/146 acute
Springfield, Mo., 1955*	4	Silo: pigeon manure	+	4/4	3/4 acute
Silva, Mo., 1955*	8	Chicken house	+	8/8	6/8 acute
Sumpter Co., Ala., 1956[23]	3	Soil	+	Not done	2/3
Hardin Co., Ohio, 1956[24]	3	Cleaning church tower; bird manure	+	Not done	3/3
Columbia Co., Wisc., 1956[25]	30	Soil; bird manure	+	20/25 Incomplete	22/25
Springfield, Mo., 1956[26]	2	Farm; ?chicken house	Not completed	2/2	2/2
Warren Co., Va., 1956[27]	6	Cleaning chicken house	Not completed	6/6	2/2
EPIDEMICS OCCURRING OUTSIDE THE UNITED STATES					
Tingo Maria, Peru[28]	6	Cave; bird guano	+	6/6	6/6
South Africa[29]	10	Exploring caves; bat guano	+ (air)	9/9	2/5
Barquisimeto, Venezuela[30]	10	Exploring cave; bat guano	+	10/10	7/10

*First reported herein.

be explained by the fact that they are exposed to heavily infected dust in a closed or semiclosed area. Aside from the size of the infecting dose, host resistance apparently plays some role in the course of the disease. Four of the five deaths reported in epidemic histoplasmosis resulted from dissemination of the disease in very young children.

In 1952, Loosli and his colleagues[15] reported the first epidemic thoroughly investigated during the acute phase of illness, with isolation of the etiologic agent from one of the patients and also from the point source of infection. Since this time five fatalities have resulted in the course of four epidemics of histoplasmosis. In four of these cases material obtained for culture both ante mortem and post mortem was positive for *H. capsulatum*. In the other case the diagnosis was made by demonstration of the organism in pathologic specimens obtained at autopsy. In the Wheaton, Maryland epidemic to be described by Kolb and Campbell[18] and the recent Wisconsin epidemic,[25] *H. capsulatum* was isolated from the sputum in four cases. Recently we have isolated the organism from two additional cases in separate epidemics. The first was an 18-year-old boy involved in the Springfield, Missouri outbreak. This patient had repeatedly negative sputum cultures early in the course. Later several positive sputums were obtained concomitantly with the "shelling" out of a pneumonic lesion. The second case was a physician who investigated the epidemic in Silva, Missouri. While collecting soil samples in a chicken coop he was unavoidably exposed to considerable dust. Although repeated sputum cultures were negative during the course of his illness, he subsequently developed a pleural effusion from which *H. capsulatum* was isolated. The recovery of the organism from 11 patients involved in 8 epidemics in the United States as well as several of those epidemics outside the United States, offers conclusive evidence for *H. capsulatum* as the etiologic agent. In 2 of these epidemics *H. capsulatum* has not been isolated from the soil.

In the past, epidemic histoplasmosis has been repeatedly referred to as a nonfatal illness. The occurrence of five fatalities in somewhat over 400 cases, or a mortality rate of 1 per cent, greatly exceeds that of endemically occurring histoplasmosis.

Roentgenologic Features.—The x-ray findings in epidemic histoplasmosis are usually striking. Originally it was considered that they were uniform. However, as a result of a number of investigations of smaller family epidemics it is now clear that the picture may vary from the mildest lymph node enlargement or the smallest detectable single pulmonary nodule to the widespread and well known "snow storm" picture. Variations include single or multiple pneumonic areas and the so-called picture of "primary atypical pneumonia." It is probable that these variations merely represent the pulmonary response to varying numbers of inhaled spores. Figs. 2 to 6 show the variety of x-ray changes seen in a single epidemic.[22] The well known "snow storm" picture apparently represents multiple foci of pneumonitis resulting from the inhalation of a large number of organisms. During the first week of illness, there is often little change except for an increase in the hilar shadows. Usually within several days, a varying number of soft irregular infiltrations can be demonstrated in the lung parenchyma. After several weeks these lesions usually become "harder" and more discrete.

Resolution usually becomes apparent by the second month but may take up to six months for complete clearing. A high percentage of these reveal calcification after several years, the multiple pneumonic foci leading to "miliary calcifications," the single lesions often resulting in a "Ghon-like" complex.

Relapses and Recurrences.—Relapses and recurrences of histoplasmosis are not unusual, although less common in the epidemic type. As a matter of fact they tend to be the rule when the patient is ambulated too early. The patient may feel well while in bed, but as soon as he is permitted activity the symptoms return; fever, cough, and prostration force the patient back to bed. The symptoms of weakness and prostration often continue for some months following an acute attack. Recurrences were originally thought to be infrequent. Recently

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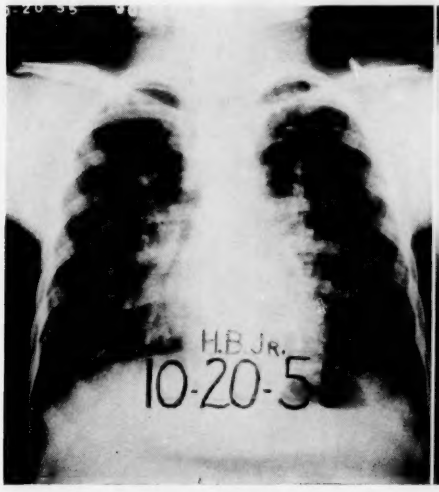

Fig. 2.

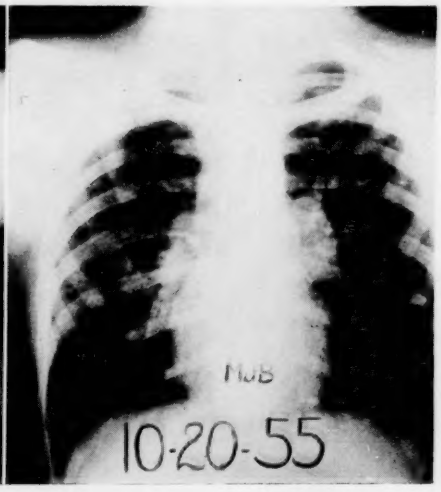

Fig. 3.

Fig. 2.—W. M., aged 9 years. Disseminated miliary infiltrations with bilateral hilar adenopathy.
Fig. 3.—W. F., aged 12 years. Marked bilateral hilar adenopathy with scattered infiltrates.

we have seen a number of cases in which bouts of pneumonia recur at intervals, sometimes of several years, following the original attack definitely diagnosed as histoplasmosis. Whether these represent recurrences or new infections is not clear, but they probably represent recurrences since they tend to occur at the same site. We are following one young patient now who has had four relapses over three years. Each relapse is accompanied by the clinical picture of pneumonia with demonstrable pulmonary infiltrations (Figs. 7 to 10).

Chronic Manifestations.—The occurrence of chronic histoplasmosis is becoming recognized with increasing frequency. It was originally thought that the patient either recovered or succumbed in fairly short order, and this probably is the general rule. However, some patients in all age groups develop the chronic

form of the disease. This is sometimes characterized by pulmonary, sometimes by systemic manifestations. We have one girl under observation and therapy who has had a large liver and spleen and positive lymph node culture for Histoplasma for almost a year. In addition, many of the older cases carry their disease

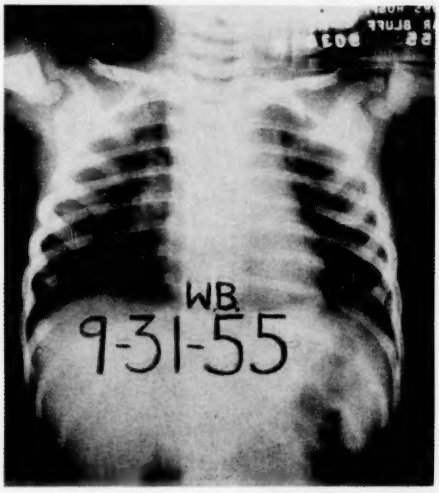

Fig. 4.—W. M., aged 1 year. Hilar and mediastinal lymphadenopathy.

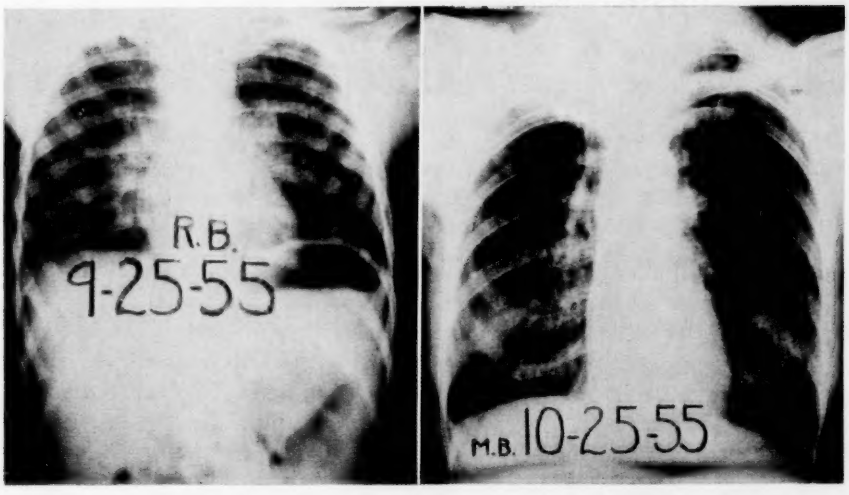

Fig. 5.—W. M., aged 6 years. Scattered pulmonary infiltrations with hilar adenopathy.

Fig. 6.—W. F., aged 54 years. Apical infiltration with probable cavity on right, uncovered during investigations of epidemic, probably of long standing. Sputum cultures negative for acid-fast bacilli, fungus cultures in progress.

in a chronic form for many months before fatal termination. The most chronic type of histoplasmosis is the cavitary form[31] which does not appear to be a common sequela of epidemic type histoplasmosis.

Another chronic complication of histoplasmosis results from the healing process. Fibrosis and calcification of the peribronchial lymph nodes may lead to

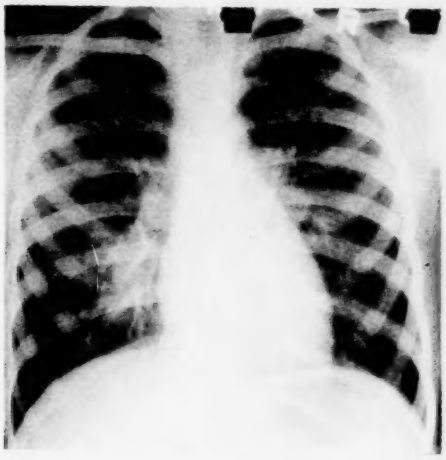

Fig. 7.—W. F., aged 6 years. Hospitalized with penumonic infiltration right base in 1951. Tuberculin skin test negative, Histoplasmin positive. Complement fixation test for histoplasmosis positive 1:64.

Fig. 8.—Recurrence of fever and cough in 1952. X-ray demonstrates spread of pneumonic process in right base with possible thin walled cavity. Enlarged left hilar lymph node. Complement fixation test positive 1:32.

constriction of a bronchus with subsequent atelectasis of a portion of the lung. With the passage of time this atelectatic area becomes superinfected with secondary invaders, and bronchiectasis develops. Usually this bronchiectasis is not accompanied by activity of the histoplasmosis, but is rather to be considered a

result of healing of the disease. We have, however, seen one case in which *H. capsulatum* was incriminated as the most probable cause of the bronchiectatic lesions.

Epidemiology.—The epidemics have furnished a unique opportunity for determining the incubation period and attack rates, as well as the clinical spectrum

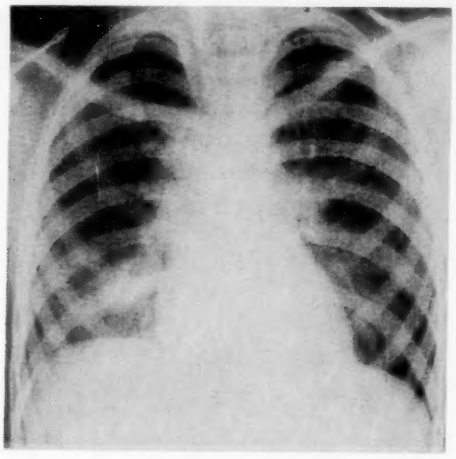

Fig. 9.—Recurrence at right base with new lesion right mediastinum in 1954. Complement fixation test positive 1:32.

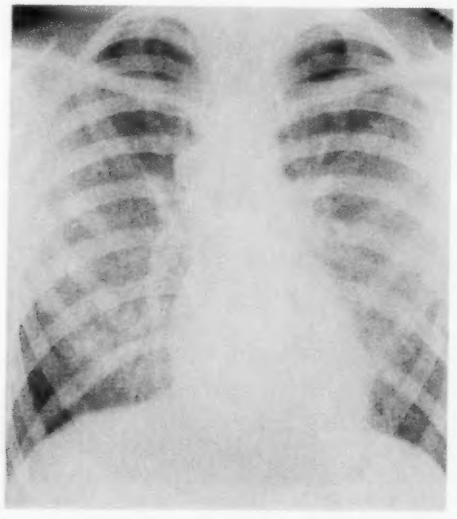

Fig. 10.—Residual fibrosis and beginning calcification right base in 1956. Large calcified supra-aortic lymph node on right. Complement fixation test negative.

of the disease. This is because the time of the exposure usually can be defined by the coincident exposure of a number of persons. From studies of this type it is quite evident that the original estimates of the incubation period of 7 to 14

days are essentially correct. Attack rates remain high (80 to 100 per cent), most of those exposed developing lung lesions and illness, with the number of lesions being roughly related to the degree of exposure.

There are a number of interesting epidemiologic implications in the epidemics. The first is the fact that most epidemics have occurred in young adults. This raises the question as to whether the disease is more severe in young adults previously uninfected, or whether it is the simultaneous onset of a number of cases in this age group that brings the epidemic to the attention of the physician. It appears unlikely that symptoms are more severe in young adults, especially when one remembers that of the five deaths which occurred in this series, four occurred in very young children. Also the occurrence of a number of epidemics involving younger children brings out the fact that epidemics may involve all ages. Another issue is whether the multiple lesions occurring in the epidemic type of disease represent blood-borne dissemination of the organism, or whether each focus represents the implantation of a spore, with subsequent inflammation. The epidemiologic evidence strongly supports the latter concept, and the correlation of the number of lesions with apparent degree of exposure to the spore-borne dust further supports this concept.

VOL.
5
1957

Public Health Implications.—Frequent confusion of the lesions found in histoplasmosis with those of tuberculosis has led to considerable difficulty in diagnosis and treatment of tuberculosis. Since treatment for tuberculosis is ineffective in histoplasmosis, it is obvious that these disease entities should be separated. Also the noncontagiousness of histoplasmosis should be recognized and cases treated accordingly when found among the general population.

One of the lessons learned from the epidemics is the potential danger to other persons of the point sources. We have had two physicians and a nurse infected while collecting soil samples at the point sources of epidemics. One of these infections was quite severe, complicated by thrombophlebitis of the femoral vein and pulmonary embolism. In two other epidemics[5,13] new infections occurred at a later period following an earlier epidemic at the same site. Accordingly care must be taken in investigation of the epidemic sites and in their eradication. The frequent implication of chicken houses, particularly unused ones,[32] presents inferences in regard to present methods of raising chickens. These methods permit accumulation of large amounts of litter in the chicken house. It is obvious that such conditions are ideal for the growth and dissemination of *H. capsulatum*. On the other hand the frequency of the isolation of the organism from chicken houses suggests practical public health methods of control: (1) the prevention of exposure to chicken houses of young children, who have excessive susceptibility; (2) the prevention of dust-borne dissemination of the organism during the cleaning of the chicken houses by wetting down the debris with water or some disinfectant; (3) the alerting of the general population, particularly the city population, to the presence of this problem. There is suggestive evidence accumulating that much of the exposure occurring among city children is due to their visits to farm areas.[33] Should this work be substantiated it would indicate further public health preventive measures.

Twelve of the 41 epidemics reported in this paper were associated with handling of chicken excreta, 7 with pigeon excreta and 3 with bat excreta. In all,

some 23 of the 41 (or 56 per cent) were associated with exposure to bird excreta. Visits to a farm, playing in a cellar, barn, or cave account for 13 more epidemics. It is obvious that these places may also have been contaminated with bird excreta.

Presence of a Positive Skin Test.—During the course of acute pulmonary infections due to *H. capsulatum*, skin sensitivity to Histoplasmin usually develops within 2 to 3 weeks after the onset of illness. Several cases in the recent epidemic in Wisconsin[25] are reported with negative skin tests which were done very early in the disease. Once sensitivity develops, present evidence indicates that this sensitivity is retained for many years and probably for the remainder of life. Hence a positive Histoplasmin reaction, like a positive tuberculin reaction, may indicate either present or past infection. Of all the persons involved in epidemics only one had a negative Histoplasmin skin test throughout the disease. This was a young boy with disseminated disease that terminated fatally. As with tuberculin an anergic response may be found during critical illness. In the group of patients tested by us, only a small percentage reacted to tuberculin, Coccidioidin, or Blastomycin. To evaluate the finding of 100 per cent positive Histoplasmin reactors, it is necessary to correlate these findings with the percentage of the general population that would be expected to react in that particular area of the country. Fig. 1 shows the location of these epidemics correlated with the geographic incidence of skin test sensitivity among surveyed populations. It should be noted that 11 of these epidemics occurred in nonendemic or "fringe areas." Two of the epidemics occurring in the "high area" of skin test sensitivity involved army personnel from nonendemic areas. The expected rate of Histoplasmin sensitivity is related not only to residence but also to age. The relatively large number of children involved in epidemics occurring in the "high area" and the lack of calcified lesions in the older patients lead us to believe that the rate of Histoplasmin positivity among the persons involved in the epidemics was in all probability low prior to the epidemic.

Although there are several instances in which exogenous reinfection has been strongly suspected but not proved, it was felt in the Arkansas epidemic[22] that a number of reinfection cases had occurred. In these patients the incubation period was considerably shortened and the illness was of a milder nature.

Serologic Tests.—Unless skin test conversion can be demonstrated during the course of illness, a positive skin test has no value in evaluating the activity of the disease process. Serologic tests used as aids in diagnosis and in assessing activity of disease have been of three types: (1) complement fixation, (2) collodion agglutination, and (3) precipitin. The C.F. test using Histoplasmin and whole or disrupted yeast-phase antigen is used most commonly. The relative value of each of these serologic tests in human disease has not been statistically substantiated. It would appear that the precipitin reaction is a more sensitive indicator of activity in early disease. Circulating antibodies are usually demonstrable within 3 to 4 weeks after the onset of illness due to *H. capsulatum*. Precipitating antibodies usually persist for a relatively short period (several months) but the C.F. antibodies are present for varying lengths of time, often for several years. There is some evidence that the level of circulating C.F. antibody is proportional to the severity of illness. In asymptomatic and mild cases, C.F.

antibodies are infrequently demonstrable. In all the epidemics considered in this paper there is serologic evidence of infection with *H. capsulatum* in each instance. Fortunately many of the investigators of the earlier epidemics in which the etiology was obscure had foresight enough to save serum from acutely ill patients. In the 27 epidemics in which serologic studies were carried out on such sera, an analysis showed that 215 of 280 cases, or 75 per cent, had positive titers. In the outbreak among Arkansas school children[22] a number of mild illnesses were uncovered as the result of survey methods. If this study is eliminated, 85 per cent of the cases in the remaining epidemics had positive serologic tests for histoplasmosis. This is certainly convincing evidence implicating *H. capsulatum* as the etiologic agent and points up the fact that epidemic histoplasmosis usually results in rather severe illness.

Soil.—The peculiar geographic distribution of histoplasmosis led a number of early investigators to implicate the soil as the probable source of infection. However, it was not until 1949 that *H. capsulatum* was isolated by Emmons[34] from soil in an environment where animal infections were occurring. In the majority of these epidemics infection was related to some activity resulting in creating dust or handling dried fowl excreta. Attempts were made to isolate *H. capsulatum* from the point source of all these epidemics. If one considers the fact that epidemiologic investigations in several of these outbreaks did not reveal the exact "point source" of infection, and that in two additional outbreaks, soil could not be obtained from the exact point source, it is indeed remarkable that positive isolations have been made in 24 of the epidemics.

Treatment of Epidemic Histoplasmosis.—It is apparent that in the past the vast majority of the cases of epidemic histoplasmosis have recovered. A mortality rate in excess of 1 per cent and the prolonged hospitalization necessary in many of the cases justifies experimental drug therapy. However, due to the infrequency of epidemics and the failure of etiologic recognition early in the course of the disease, such therapeutic trials as we have undertaken have been mostly in the chronic cavitary type of disease in which therapeutic response is difficult to evaluate. We have now treated almost 50 cases of histoplasmosis with various chemicals and antibiotics, and of those employed, two appear to have promise.[35] The two experimental drugs which show promise are Amphotericin B* (an antibiotic manufactured by E. R. Squibb and sons), and Amebacide* (a chemical [bistertiary amine] manufactured by Eli Lilly and Company). On the basis of results obtained with these drugs we intend to continue their use in further studies. It would certainly appear indicated to attempt therapeutic trials in severe cases of epidemic histoplasmosis. We have supplies of these drugs and have set up protocols for evaluating treatment. We shall be happy to collaborate with physicians in such studies.

SUMMARY

Clinical, roentgenologic, and epidemiologic features of 41 epidemics of histoplasmosis are reviewed. The problems of recurrences and chronic manifestations are briefly discussed and the need for adequate drug therapy emphasized.

*The use of trade names is for identification only, and does not constitute endorsement by the Public Health Service.

The occurrence of epidemics outside the United States, two in South America and several in South Africa, indicates that the problem of histoplasmosis is not limited to this continent. Although it is unquestionably true that knowledge of histoplasmosis and its prevalence is most advanced in this country, cases have been reported from almost every country in the world. Whether the epidemiologic factors involved in the spread of this disease are similar in different parts of the world remains to be established. However, the size of this problem, as outlined in the introduction, clearly indicates its importance to the medical profession in this country.

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VOL. 5 195

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OL.
5
957